1246872

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

November 09, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/507,427*
FILING DATE: *September 30, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/32289*

BEST AVAILABLE COPY

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

BEST AVAILABLE COPY

48003-3.JBM.242823

WENMM SB/16 (10-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

22154 U.S. PTO 60/507427 09/30/03

17224 U.S. PTO 09/30/03

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.	EL 984270653 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Joseph R.	Garlich	Westfield, Indiana

☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number: 30565

Type Customer Number here

OR

☐ Firm *or* Individual Name	Woodard, Emhardt, Moriarty, McNett & Henry LLP				
Address	Bank One Center/Tower				
Address	111 Monument Circle, Suite 3700				
City	Indianapolis	State	Indiana	ZIP	46204-5137
Country	U.S.A.	Telephone	(317) 634-3456	Fax	(317) 637-7561

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification *Number of Pages* 198 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* Ø ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (*check all that apply*)

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 23-3030

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: National Cancer Institute, 1R41CA92835-01

Respectfully submitted,

SIGNATURE James B. Myers, Jr.

TYPED or PRINTED NAME James B. Myers, Jr.

TELEPHONE (317) 634-3456

Date 09/30/2003

REGISTRATION NO. (if appropriate) 42,021

Docket Number: 48003-3

Express Mail Label No.	*EL 984270653 US*	Date of Deposit	09/30/2003

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Mail Stop Provisional Patent Application, Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.

Signature Laurie A. Epler

Expiration Date 8/31/2001

Department of Health and Human Services
Public Health Service

Small Business Technology Transfer Program

Phase I Grant Application

Follow instructions carefully.

Leave blank — for PHS use only.		
Type	Activity	Number
Review Group		Formerly
Council Board *(Month, year)*		Date Received

1. TITLE OF APPLICATION *(Do not exceed 56 typewriter spaces)*
Chelate Based Scaffolds (Chelabody) In Tumor Targeting

2. SOLICITATION NO. PHS 2000-2 PAR-00-030 F.L.A.I.R. ☐ New Investigator

3. PRINCIPAL INVESTIGATOR

3a. NAME *(Last, first, middle)*
Joseph R. Garlich

3b. DEGREE(S) B.A. Ph.D.

[redacted]

3d. POSITION TITLE
Principal Investigator

3e. MAILING ADDRESS *(Street, city, state, zip code)*
9731 Trilobi Drive
Indianapolis, IN 46236

3f. TELEPHONE AND FAX *(Area code, number, and extension)*
TEL: 317-581-1635
FAX: 317-823-7552

BITNET/INTERNET Address:
joegarlich@aol.com

4. HUMAN SUBJECTS ☒ NO ☐ YES
4a. If "Yes," Exemption no. ☐ or IRB approval date ☐ ☐ Full IRB or ☐ Expedited Review
4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS ☐ NO ☒ YES
5a. If "Yes," IACUC approval date: 10/4/99
5b. Animal welfare assurance no.: A3231

6. DATES OF PROJECT PERIOD
From: [redacted] Through: [redacted]

7. COSTS REQUESTED
7a. Direct Costs: $ $501,277
7b. Total Costs: $ 501,277

8. PERFORMANCE SITES *(Organizations and addresses)*
Dept. of Med. Chem & Mol. Pharmacology
Purdue University
1333 Pharmacy Building; Room 308
West Lafayette, IN 47907-1333

ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

9. APPLICANT ORGANIZATION *(Name and address of applicant small business concern)*
ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

10. ENTITY IDENTIFICATION NUMBER #35-2100628
Congressional District 6

11. SMALL BUSINESS CERTIFICATION
☒ Small Business Concern ☐ Women-owned
☐ Socially and Economically Disadvantaged

12. NOTICE OF PROPRIETARY INFORMATION: The information identified by asterisks(*) on pages 18,19,20,21,22,23,24 of this application constitutes trade secrets or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that such information shall be used or disclosed only for evaluation of this application, provided that, if a grant is awarded as a result of or in connection with the submission of this application, the Government shall have the right to use or disclose the information herein to the extent provided by law. This restriction does not limit the Government's right to use the information if it is obtained without restriction from another source.

13. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title only of your proposed project, and the name, address, and telephone number of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information or possible investment? ☒ YES ☐ NO

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Barry A. Dreikorn, Ph.D.
Title: Executive Vice President
Address: 9731 Trilobi Drive
Indianapolis, IN 46236

Telephone: 317-823-0732
FAX: 317-823-7552
BITNET/INTERNET Address:
comchemtech@home.com

15. PRINCIPAL INVESTIGATOR ASSURANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a *(In ink. "Per" signature not acceptable.)*
Joseph R Garlich

DATE [redacted]

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF PERSON NAMED IN 14 *(In ink. "Per" signature not acceptable.)*
Barry Dreikorn

DATE [redacted]

Abstract of R search Plan

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236
317-823-0732

YEAR FIRM FOUNDED 2000

NO. OF EMPLOYEES (include all affiliates) 3

TITLE OF APPLICATION Chelate Based Scaffolds (Chelabody) In Tumor Targeting

KEY PERSONNEL ENGAGED ON PROJECT

NAME	ORGANIZATION	ROLE ON PROJECT
Joseph R. Garlich	ComChem Technologies, Inc.	Principal Investigator
TBA	ComChem Technologies, Inc.	Reseach Scientist
TBA	ComChem Technologies, Inc.	Senior Research Scientist
Mark Green	Purdue University	Co-Investigator
Carla Mathias	Purdue University	Project Coordinator
TBA	Purdue University	Post-doc Researcher

ABSTRACT OF RESEARCH PLAN: State the application's broad, long-term objectives and specific aims, making reference to the health-relatedness of the project. Describe concisely the research design and methods for achieving these goals and discuss the potential of the research for technological innovation. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. *Therefore, do not include proprietary or confidential information.* DO NOT EXCEED 200 WORDS.

The current paradigm in therapeutic nuclear medicine is to optimize receptor binding molecules and then add on a moiety capable of carrying a radioisotope. This "afterthought" modification process results in suboptimum performance for such agents when dealing with molecules smaller than monoclonal antibodies.

A new concept proposed here is to utilize the properties of chelating agents to build in the desired recognition functionalities. The conformationally restricted metal-ligand complexes proposed herein offer the opportunity to attach molecular recognition units in a certain three-dimensional spatial arrangement that will allow the molecule to mimic protein-protein (or peptide-receptor) binding interactions such as those found in antibody-antigen recognition.

Synthetic molecules that mimic antibody-antigen recognition are known as chemobodies. The new approach in this proposal gives rise to a subset of chemobody molecules hereby termed chelabodies to reflect the critical role that the conformationally restricted metal-ligand complex plays in creating the molecular recognition event.

This concept presented here is broadly applicable to receptors in general but will focus on designing (molecular modeling), synthesizing (through combinatorial methodology), screening (*in vitro, in vivo* in tumor-bearing mice) and optimizing metal-ligand complex-based antagonists of the $\alpha_v\beta_3$ receptor that will deliver therapeutic radioactive metal ions to the neovasculature of $\alpha_v\beta_3$ receptor-positive cancers.

Provide key words (8 maximum) to identify the research or technology.

Combinatorial, chelabody, anticancer , complex, chelating agents, integrins, radioisotope

Provide a brief summary of the potential commercial applications of the research.

The proposed work is aimed at the discovery, optimization and initial development of a tumor localizing therapeutic radiopharmaceutical drug that targets $\alpha_v\beta_3$ receptors in new blood vessels required for tumor growth. The methodology proposed (combinatorial chelating agent synthesis methodology) is likely to be broadly applicable to address other target receptors.

Year 1

Principal Investigator (Last, first, middle): Garlich, Joseph R.

FROM [redacted] TO [redacted]

Budget of Applicant Organization for Phase I—Direct Costs Only

PERSONNEL (Applicant organization only) NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED (omit cents) Salary Requested	Fringe Benefits	TOTALS
Joseph R. Garlich, Ph.D.	P.I	12	15	120,000	0	0	0
TBA, M.S.	Research Scientist	12	100	72,000	72,000	14,400	86,400
TBA, Ph.D	Senior Res. Scientist	12	25	96,000	15,000	3,000	18,000
SUBTOTALS →					87,000	17,400	104,400

CONSULTANT COSTS 2 Consultants X 6 days X $1,000 per day = $6,000	6,000
EQUIPMENT (Itemize)	
SUPPLIES (Itemize by category) $13,000 Chemicals and combinatorial chemistry supplies $ 3,000 Glassware	16,000
TRAVEL	
PATIENT CARE COSTS — Inpatient	
PATIENT CARE COSTS — Outpatient	
CONTRACTUAL COSTS Subcontract with Dr. Mark Green of Purdue University composed of $81,494 in Direct costs and $42,377 in indirect costs. (see attached budget sheets)	123,871
OTHER EXPENSES (Itemize by category)	
TOTAL DIRECT COSTS (Also enter on Face Page, Item 7a) →	$250,271
FIXED FEE REQUESTED	$ 0

OTHER SUPPORT (see instructions) ☐ NO ☐ YES

Year 2

Principal Investigator (Last, first, middle): Garlich, Joseph R.

Budget of Applicant Organization for Phase I—Direct Costs Only

FROM [redacted] TO [redacted]

PERSONNEL (Applicant organization only) NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED (omit cents) Salary Requested	Fringe Benefits	TOTALS
Joseph R. Garlich, Ph.D.	P.I	12	15	127,200	0	0	0
TBA, M.S.	Research Scientist	12	100	76,320	76,320	15,264	91,584
TBA, Ph.D	Senior Res. Scientist	12	25	101,760	10,000	2,000	12,000
SUBTOTALS					86,320	17,264	103,584

CONSULTANT COSTS 1 Consultant X 3 days X $1,000 per day = $3,000	3,000
EQUIPMENT (Itemize)	
SUPPLIES (Itemize by category) $13,000 Chemicals and combinatorial chemistry supplies $ 3,000 Glassware	16,000
TRAVEL	
PATIENT CARE COSTS — Inpatient / Outpatient	
CONTRACTUAL COSTS Subcontract with Dr. Mark Green of Purdue University composed of $84,488 in Direct costs and $43,934 in indirect costs (see attached budget sheets).	128,422
OTHER EXPENSES (Itemize by category)	
TOTAL DIRECT COSTS (Also enter on Face Page, Item 7a)	$ 251,006
FIXED FEE REQUESTED	$ 0

OTHER SUPPORT (see instructions) [X] NO [ ] YES

Principal Investigator: Garlich, Joseph R.

PURDUE ~~YEAR 1~~

Budget of Research Institution for Phase I

FROM: [redacted] | TO: [redacted]

NAME AND ADDRESS OF RESEARCH INSTITUTION
Purdue University, West Lafayette, IN

PERSONNEL

NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED (omit cents): Salary Requested	Fringe Benefits	TOTALS
Mark Green, Ph.D.	P.I.	12	10	108,675	10,913	3,192	14,105
TBA, Ph.D.	Post-doc Researcher	12	100	30,000	30,000	5,700	35,700
Carla Mathias	Project Cood.	12	5	60,944	3,057	632	3,689
V. Joe Davisson, Ph.D.	Collabo	12	5	0	0	0	0
SUBTOTALS					43,970	9,524	53,494

CONSULTANT COSTS	
EQUIPMENT (itemize)	
SUPPLIES (itemize by category) $18,000 Animal studies, Isotope procurement $10,000 Assay costs, disposables, solvents, counting supplies	28,000
TRAVEL	
PATIENT CARE COSTS: Inpatient / Outpatient	
CONTRACTUAL COSTS	
OTHER EXPENSES (itemize by category)	
TOTAL DIRECT COSTS	$ 81,494
INDIRECT COSTS (show calculation) 81,494 X 0.52 = 42,377	42,377
TOTAL COSTS (Also enter as "Contractual Costs" on Budget of Applicant Organization—form page 3)	$ 123,871

CERTIFICATION OF RESEARCH INSTITUTION PARTICIPATION

Through the signature below of the duly authorized representative of the research institution on this budget page, and by way of the signature of the official signing for applicant organization (small business concern) on the Face Page of the application, the small business concern and the research institution certify jointly that: (1) the proposed STTR project will be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("cooperative research and development"); (2) the proposed STTR project is a cooperative research or research and development effort to be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("performance of research and analytical work"); and (3) regardless of the proportion of the proposed project to be performed by each party, the small business concern will be the primary party that will exercise management direction and control of the performance of the project. If the research institution is a contractor-operated federally funded research and development center, the duly authorized representative of the contractor-operated federally funded research and development center certifies, additionally, that it (4) is free from organizational conflicts of interest relative to the STTR program; (5) did not use privileged information gained through work performed for an STTR agency or private access to STTR agency personnel in the development of this STTR grant application; and (6) used outside peer review, as appropriate, to evaluate the proposed project and its performance therein.

SIGNATURE of duly authorized representative	Typed Name	Title	Date
Diane Troyer	Diane Troyer	Assist Dir Spon ~~Program Administration~~	[redacted]

PHS 6246-1 (Rev. 1/96)

PURDUE YEAR # 2 Principal Investigator: Garlich, Joseph R.

Budget of Research Institution for Phase I	FROM: [redacted]	TO: [redacted]

NAME AND ADDRESS OF RESEARCH INSTITUTION
Purdue University, West Lafayette, IN

PERSONNEL

NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	Salary Requested	Fringe Benefits	TOTALS
Mark Green, Ph.D.	P.I.	12	10	114,109	11,458	3,351	14,809
TBA, Ph.D.	Post-doc Researcher	12	100	31,800	31,800	6,042	37,842
Carla Mathias	Project Cood.	12	5	63,382	3,180	657	3,837
V. Joe Davisson, Ph.D.	collabor t	12	5	0	0	0	0
SUBTOTALS →					46,438	10,050	56,488

CONSULTANT COSTS	
EQUIPMENT (itemize)	
SUPPLIES (itemize by category) $18,000 Animal studies, isotope procurement $10,000 Assay costs, disposables, solvents, counting supplies	28,000
TRAVEL	
PATIENT CARE COSTS — Inpatient / Outpatient	
CONTRACTUAL COSTS	
OTHER EXPENSES (itemize by category)	
TOTAL DIRECT COSTS	$ 84,488
INDIRECT COSTS (show calculation) 84,488 X 0.52= 43,934	$ 43,934
TOTAL COSTS (Also enter as "Contractual Costs" on Budget of Applicant Organization—form page 3)	$ 128,422

CERTIFICATION OF RESEARCH INSTITUTION PARTICIPATION

Through the signature below of the duly authorized representative of the research institution on this budget page, and by way of the signature of the official signing for applicant organization (small business concern) on the Face Page of the application, the small business concern and the research institution certify jointly that: (1) the proposed STTR project will be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("cooperative research and development"); (2) the proposed STTR project is a cooperative research or research and development effort to be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("performance of research and analytical work"); and (3) regardless of the proportion of the proposed project to be performed by each party, the small business concern will be the primary party that will exercise management direction and control of the performance of the project. If the research institution is a contractor-operated federally funded research and development center, the duly authorized representative of the contractor-operated federally funded research and development center certifies, additionally, that it: (4) is free [illegible] [illegible] relative to the STTR program; (5) did not use privileged information gained through work performed for an STTR agency or private access to STTR agency personnel in the development of this STTR grant application; and (6) used outside peer review, as appropriate, to evaluate the proposed project and its performance therein.

SIGNATURE of duly authorized representative	Printed Name	Title	Date
[signature]	Diane Troyer	Assist Dir Sp n Pro Ad	11/20/00

Budget Justification

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify costs accordingly.

PERSONNEL:

Applicant Organization:

Joseph R. Garlich, Ph.D., Principal Investigator, will contribute 15% of his time (and no salary as his compensation be leverage money supplied by CCTI) and will assist in the experimental design and implementation of synthetic work, both traditional and combinatorial (solid-phase) and supervise and coordinate the experimental studies. Will also be responsible jointly with Dr. Green for interpretation of the data and providing project direction.

TBA, Ph.D., Senior Research Associate., will be skilled in organic synthesis (solution and solid phase) and have molecular modeling expertise. This position will contribute 25% of time (but only receive 16% of salary with the remainder compensation as leverage money from CCTI) to the project performing hands-on solid phase synthesis, experimental design, and molecular modeling studies.

TBA, M.S., Research Associate, will be skilled in organic synthesis (solution and solid phase) with some experience in complexation chemistry and will be well versed in analytical instrumentation and purification methods. Will be responsible for developing solid phase protocols and production and purification of combinatorial libraries of target molecules.

Donald Durden, M.D., Ph.D. will serve as a consultant with an emphasis on bioassays, interpretation of results, biochemical pathways,and expert on human neovasculature.

Marty O'Donnell, Ph.D., (Professor, Chemistry Department, IUPUI) will serve as a consultant and will assist in the synthesis experimental design including solid-phase synthesis approaches to make unnatural amino acids.

Research Institution:

Dr. Mark Green, Ph.D., Co-Investigator, will contribute 10% of his time in the experimental design of the project including the bioassays, radioisotope labeling, and animal biodistribution work as well as interpretation of the experimental results.

TBA, Ph.D., Post-Doctoral research associate conducting biochemistry and medium-throughput bioassays, experimental design, performing animal biodistribution studies, data collection and presentation and interpretation.

(CONTINUED ON NEXT PAGE)

Resources

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. (The research to be performed by the applicant small business concern and its collaborators must be in facilities that are available to and under the control of each party for the conduct of each party's portion of the proposed project.) Indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant small business concern and any other performance site listed on the FACE PAGE. Identify support services such as secretarial, machine shop, electronics shop, and the extent to which they will be available to the project. Use continuation page(s) if necessary.

Research Institution:

Purdue University, resides on a 1556 acre main campus in West Lafayette Indiana less than a 2 hour drive from the Indianapolis facility of CCTI. The Department of Medicinal Chemistry and Molecular Pharmacology is in the School of Pharmacy and Pharmacal Sciences. The department occupies over 341,000 sq. ft. of space; over 20,000 sq. ft. are devoted to research. Major shared instrumentation and facilities are available within the School of Pharmacy. The Combinatorial Chemical Biology Center is in the same building and will be a resource for the biological screening. There is also a wide array of supporting chemical and radioanalytical equipment in Professor Green's (co-investigator) research laboratories. This equipment includes a Packard 5530 large vial (28mm) automatic gamma counting system with 3x3.25 inch NaI crystal and three user-definable counting windows; Berthold Tracemaster 20 Automatic TLC linear analyzer, two Capintec-CRC-12R radionuclide dose calibrators, Ranin Rabbitt-HP ternary gradient HPLC system equipped with Knaur variable wavelength UV/VIS

(CONTINUED ON NEXT PAGE)

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Research Institution: NMR (multinuclear Varian VXR-500 MHz, Bruker ARX 300 MHz); Mass Spectroscopy (MAT L95 HRMS, Finnigan 4000 for EI/CI, and Thermoquest LCQ with electrospray and LC/MS/MS); Beckman DU7-HS U.V. spectrometer; Nicolet FT-IR; and several scintillation counters. The Combinatorial Chemical Biology Center houses a Tecan Spectrafluor Plus, BioImage Intelligent Quantifier (IQ) for blot analysis and colony counting, PDI Discovery Series Scanner.Densitomer, Beckman LS1801 beta counter, Packard Top-Count microplate scintillation and luminescence counter, and Molecular Dynamics STORM Phosphorimager.

(CONTINUED ON NEXT PAGE)

BUDGET JUSTIFICATION (Continuation Page)

PERSONNEL(CONTINUED):

Research Institute (continued):

Carla Mathias, B.A., Project Coordinator, will serve, by benefit of extensive radiopharmaceutical laboratory experience, as coordinator and participant in the design and implementation of the proposed radiochemistry studies.

V. Jo Davisson, Ph.D., Consultant, will contribute his expertise and guidance (at no cost to project and leveraged funds from Purdue) as co-director of Purdue's Combinatorial Chemical Biology Center to set-up and run medium-throughput biochemical assays at the Center. Dr. Davisson, Professor, Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue University has many years experience in the field of biochemistry and will be responsible for integrating this proposed work into the Combinatorial Chemical Biology Center's capabilities.

RESOURCES (CONTINUED):

FACILITIES (CONTINUED):

Research Institute (continued):

detector and Canberra gamma detector system; BAS 100A electrochemical analyzer; Brinkman variable-speed ultracentrifuge; Harvard Apparatus infusion/withdrawal syringe pump Model 22; Gilson automatic fraction collector and peristaltic pump; and E-C Apparatus low voltage electrophoresis power supply.

Applicant Organization: As a start-up company ComChem Technologies (CCTI), located in the Indianapolis area is a short (<2 hour) drive to the Research Institute collaborator facility. CCTI will have in place at its facility standard synthesis equipment but more importantly will have equipment for combinatorial chemical synthesis (solution and solid-phase), both protocol development and library production tools (parallel reaction equipment, automated LCMS, software, liquid handler, etc.).

MAJOR EQUIPMENT (CONTINUED):

Applicant Organization: As a start-up company ComChem Technologies will have in place the following major equipment: 300 MHz multinuclear spectrometer, a high througput HPLC coupled with mass detector (Gilson Nebula Series), liquid handler, Argonaut Quest 210 parallel reactor , automated purification system, Irori miniKan library synthesis equipment cluster, vacuum concentrator, Infrared spectrophotomer.

Joseph R. Garlich

President and Chief Scientific Officer
ComChem Technologies Inc.

Education:

Institute and Location	Degree	Year(s)	Field of Study
University of Missouri, Columbia, MO	BA	1974-78	Chemistry
University of Missouri, Columbia, MO	BA	1974-78	Biology
University of Missouri, Columbia, MO	Ph.D.	1978-82	Organic Chemistry
University of Florida, Gainesville, FL	(Post-Doc)	1982-84	Medicinal Chemistry

Professional Experience:

2000-present	President, founder, and Chief Scientist of ComChem Technologies, Inc., Indianapolis, IN. Involved in drug disovery and development using combinatorial chemistry.
1997-2000	Research Scientist, Combinatorial Chemistry-Lead Generation, DowAgroSciences, Indianapolis, IN.
1995-1997	Research Scientist, Discovery Research Department, DowElanco, Indianapolis, IN.
1993-1995	Research Associate, Designed Chemicals R & D Department, Dow Chemical Company, Freeport, TX.
1990-1993	Research Leader, Designed Chemicals R & D Department, Dow Chemical Company, Freeport, TX.
1987-1990	Project Leader, Functional Chemicals Research Department, Dow Chemical Company, Freeport, TX.
1984-1987	Senior Research Chemist, Organic Process Research Department, Dow Chemical Company, Freeport, TX.

Honors and Awards:

1992 Gulf Coast Scientists Texas Inventor of the Year Award, Dow Chemical
1992 Gulf Coast Scientists Award For Excellence in Science, Dow Chemical
1997 DowElanco Discovery Recognition Award for Excellence in Problem Solving

SELECTED BIBLIOGRAPHY:

DeAmicis, C.V., Dripps, J.E., Garlich, J.R., Hatton, C.H., Hill, R.L. "Photochemical Stablility of Spinosad and Semi-synthetic Spinosyn Derivatives" J. Agr. Food Chem. Submitted.

Crouse, G.D., Sparks, T.G., Schoonover, J., Gifford, J., Dripps, J., Bruce, T., Larson, L.L., Garlich, J., Hatton, C., Hill, R.L., Worden, T.V., Martynow, J.G. "Recent Advances in the Chemistry of Spinosyns", Pest Management Science, in press, January 2001.

Kleschick, W.A., Davis, L.N., Dick, M.R., Garlich, J.R., Martin, E.J., Orr, N.,Ng, S.C., Pernich, D.J., Unger, S.H., Watson, G.B., Zuckermann, R.N., "The Application of Combinatorial Chemistry in Agrochemical Discovery", American Chemical Society Symposium Series; Pesticide Science: New Chemistry, in press.

Garlich, J.R., Ritzler, S.J. "Novel Nucleophilic Cleavage Agents", Poster presented at the 5th Annual High Throughput Synthesis Symposium, San Diego, CA., February 11, 2000.

Invited Seminar, IUPUI Department of Chemistry, "Combinatorial Chemistry Applications in Agrochemical Discovery", January 26, 2000.

Garlich, J.R., "Studies and Analogs of a Triglycine Lead Molecule", poster presented to the 37th National Organic Chemistry Symposium, Madison, Wisconson, June 14, 1999.

Bayouth, J., Macey, D., Kasi, L., Garlich, J., McMillan, K., Dimopoulos, M., Champlin, R., "Pharmacokinetics, Dosimetry and Toxicity of Holmium-166-DOTMP for Bone Marrow Ablation in Multiple Myeloma", Journal of Nuclear Medicine, Volume 36, pp. 730-737, 1995.

Champlin, R., Dimopoulos, M., Bayouth, J., Macey, D., Kasi L., Przepiorka, D., Pololoff, D., Garlich, J., Simon, J., Alexanian, R. "Holmium-166 DOTMP, A Bone Seeking Radiochelate For Selective Marrow Radiotherapy With Bone Marrow Transplantation (BMT) For Multiple Myeloma",

presented by Dr. Champlin at the International Society of Experimental Hematology, Rotterdam, September 1993

Ghiron, J., Volkert, W.A., Garlich, JR., "Determination of Lesion to Normal Bone Uptake Ratios of Skeletal Radiopharmaceuticals by QARG", Nuclear Medicine and Biology, Volume 18, pp. 235-240, 1991.

Parks, N.J., Kawakami, T.G., Homoff, W., Fisher, P., Garlich, J.R., Simon, J., and Champlin, R., "Bone Marrow Transplantation in Dogs After Radioablation with a Ho-166 Amino Phosphonic Acid Bone-Seeking Agent (DOTMP) ", Blood, Volume 82, pp 318-325, 1993.

Garlich, J.R., " ^{166}Ho-DOTMP: ~~A New~~ Agent For Bone Marrow Ablation" Presented at the Fortieth Annual Meeting of the Society of Nuclear Medicine, June 8, 1993, Toronto, Canada.

Garlich, J.R., "Chemistry of Novel Macrocyclic Aminophosphonic Acid Chelates of Rare Earth Radionuclides and Their In-Vivo Biodistribution". Presented at the Fortieth Annual Meeting of the Society of Nuclear Medicine, June 8, 1993, Toronto, Canada.

ISSUED UNITED STATES PATENTS:

1. Bone Marrow Suppressing Agents 4,882,142 (11/21/89)
2. Method For Purifying Aminomethylenephosphonic Acids for Pharmaceutical Use. 4,937,333 (6/26/90)
3. Bone Marrow Suppressing Agents. 4,976,950 (12/11/90)
4. Macrocyclic Aminophosphonic Acid Complexes For the Treatment of Calcific Tumors. 5,059,412 (10/22/91)
5. Macrocyclic Aminophosphonic Acid Complexes, Their Formulations and Use. 5,064,633 (11/12/91)
6. Radiolabeled Metal-Binding Protein for the Treatment of Arthritis. 5,133,956 (7/28/92)
7. Oral Compositions for Suppressing Mouth Odors. 5,286,479 (2/15/94)
8. Organic Amine Phosphonic Acid Complexes for the Treatment of Calcific Tumors. 5,300,279 (4/5/94)
9. Phytate Antimicrobial Compositions in Oral Care Products. 5,300,289 (4/5/94)
10. Method of Treating and/or Diagnosing Soft Tissue Tumors. 5,308,606 (5/3/94)
11. Oral Compositions for Inhibiting Calculus Formation. 5,318,772 (6/7/94)
12. Oral Compositions for Inhibiting Plaque Formation. 5,320,829 (6/14/94)
13. Complexes Possessing Ortho Ligating Functionality. 5,342,604 (8/30/94)
14. Radioactive Compositions for Soft Tissue Tumors. 5,342,925 (8/30/94)
15. Macrocyclic Conjugates and Their Use as Diagnostic and Therapeutic Agents. 5,435,990 (7/25/95)
16. Macrocyclic Ligands and Complexes. 5,652,361 (7/29/97)
17. Complexes Possessing Ortho Ligating Functionality and Complexes Thereof. 5,696,239 (12/9/97)
18. Conjugates Possessing Ortho Ligating Functionality. 5,714,631 (2/3/98)
19. Bicyclopolyazamacrocyclophosphonic Acid Complexes for use as Contrast Agents. 5,739,294 (4/14/98)
20. Bicyclopolyazamacrocyclophosphonic Acid Half Esters. 5,750,660 (5/12/98)
21. Macrocyclic Tetraazacyclododecane Conjugates and Their Use as Diagnostic and Therapeutic Agents. 5,756,065 (5/26/98)
22. Frozen Radiopharmaceutical Formulations. 5,762,907 (6/9/98)

PUBLISHED PENDING FOREIGN PATENT APPLICATIONS:

1. Carbonyl-Containing Degradable Chelants, Uses, and Compositions Thereof (EP-522547-A2; 1/13/93).
2. Targeted Delivery of Growth Factors for Bone Regeneration (PCT Int. Appl. WO 94/00145, 1/6/94).
3. Bicyclopolyazamacrocyclophosphonic Acids, Their Complexes and Conjugates, for use as Contrast Agents, and Processes for their Preparation (WO 94/26754. 11/24/94).

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Mark A. Green	Professor of Medicinal Chemistry

EDUCATION *(Begin with baccalaureate or other initial professional education,. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Rose-Hulman Institute of Technology, Terre Haute, Indiana	B.S.	1978	Chemistry
Indiana University, Bloomington, Indiana	Ph.D.	1982	Inorganic Chemistry
Washington University, St. Louis, Missouri	Postdoctoral	1982-85	Radiopharmaceutical Chem.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to those publications most pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Positions:

9/78-8/82	Associate Instructor and Research Associate, Department of Chemistry, Indiana University, Bloomington, IN. Research advisor: Professor Kenneth G. Caulton.
8/82-6/85	Postdoctoral Research Associate with Professor Michael J. Welch, Department of Radiology, Washington University School of Medicine, St. Louis, Missouri.
7/85-7/87	Assistant Professor, Department of Radiology, University of Minnesota Medical School, Minneapolis, Minnesota. Joint appointment, College of Pharmacy, Department of Medicinal Chemistry.
7/87-6/90	Assistant Professor of Nuclear Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.
3/90-present	Adjunct Faculty Appointment, Department of Radiology, Indiana University School of Medicine, Indianapolis, Indiana.
7/90-6/94	Associate Professor of Medicinal Chemistry, Division of Nuclear Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.
7/94-present	Professor of Medicinal Chemistry, Division of Nuclear Pharmacy, Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.

Awards and Other Professional Activities:

Twelfth Tetalman Memorial Award, The Society of Nuclear Medicine, 1992
NIH Research Career Development Award, from the National Heart, Lung, and Blood Institute, 8/86-7/91; Tau Beta Pi, 1977
American Chemical Society, 1977-present; Society of Nuclear Medicine, 1983-present; Sigma Xi, 1988-present; International Society of Cerebral Blood Flow and Metabolism, 1991-present; Institute for Clinical PET, 1991-present
American Association for Cancer Research, 1997-present. Society for Nuclear Imaging in Drug Development, 2000-present.

Most Recent Publications Relevant To This Proposal (from a total of 92):

"Synthesis of Compound Libraries Based on 3,4-Diaminocyclopentanol Scaffolds," *J. Comb. Chem.*, 2:297-300; 2000. Y. Guan, M.A. Green, and D.E. Bergstrom.

"Novel gallium(III) complexes transported by MDR1 P-glycoprotein: potential PET imaging agents for probing P-glycoprotein-mediated transport activity *in vivo*," *Chemistry and Biology*, 7:335-343; 2000. V. Sharma, A. Beatty, S.P. Wey, L. Bass, C.L. Crankshaw, M.A. Green, M.J. Welch, and D. Piwnica-Worms.

"Synthesis of [^{99m}Tc]-Tc-DTPA-Folate and Its Evaluation as a Folate-Receptor-Targeted Radiopharmaceutical," *Bioconjugate Chemistry* 11:253-257; 2000. C.J. Mathias, D. Hubers, P.S. Low, and M.A. Green.

"A Kit Formulation for Preparation of [^{111}In]In-DTPA-Folate, a Folate-Receptor-Targeted Radiopharmaceutical," *Nucl. Med. Biol.*, 25:585-587; 1998. C.J. Mathias and M.A. Green.

"Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts," *Nucl. Med. Biol.*, 26:23-25; 1999. C.J. Mathias, S. Wang, P.S. Low, D.J. Waters, and M.A. Green.

"Evaluation of ^{111}In-DTPA-Folate as a Potential Folate-Receptor-Targeted Radiopharmaceutical," *J. Nucl. Med.*, 39:1579-1585; 1998. C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, and M.A. Green.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Carla J. Mathias	Project Coordinator

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
DePauw University, Greencastle, Indiana	B.A.	1976	Zoology & Chemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

Professional Positions:

12/77 - 10/78 Research Technician I, Hemostasis and Thrombosis Research, with H. J. Joist, M.D., Washington University School of Medicine, St. Louis, Missouri.

7/78 - 6/86 Senior Research Technician, Nuclear Medicine Research, with M. J. Welch, Ph.D. and B. A. Siegel, M.D., Washington University School of Medicine, St. Louis, Missouri.

7/86 - 6/89 Research Assistant, Division of Radiation Sciences, with M. J. Welch, Ph.D., Washington University School of Medicine, St. Louis, Missouri.

7/89 - 6/90 Research Associate, Division of Radiation Sciences, with M. J. Welch, Ph.D., Washington University School of Medicine, St. Louis, Missouri

1/91 - 8/94 Visiting Research Instructor, Department of Medicinal Chemistry, School of Pharmacy, Purdue University, West Lafayette, Indiana

7/94 - 6/95 Project Coordinator, Purdue National Biomedical Tracer Facility Project, Purdue University, West Lafayette, Indiana

6/96 - present Research Project Coordinator, Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy, Purdue University, West Lafayette, Indiana

Awards and Other Professional Activities:

Missouri Valley Chapter-Society of Nuclear Medicine, Young Investigator Award, Runner-up, 1979-1981;Young Investigator Award, 1982

National Science Foundation, Travel Award, to N.A.T.O. Advanced Studies Institute, Greece, 6/87

Society of Nuclear Medicine, Berson-Yalow Award (annual award for outstanding paper in the application of radioisotope techniques in receptor or immunoassay), Co-awardee in both 1988 and 1990.

Relevant Publications (selected from a total of 85):

C.J. Mathias, D. Hubers, P.S. Low, and M.A. Green. Synthesis of [^{99m}Tc]-Tc-DTPA-Folate and Its Evaluation as a Folate-Receptor-Targeted Radiopharmaceutical," *Bioconjugate Chemistry* 11:253-257; 2000.

C.J. Mathias and M.A. Green. A Kit Formulation for Preparation of [^{111}In]In-DTPA-Folate, a Folate-Receptor-Targeted Radiopharmaceutical, *Nucl. Med. Biol.*, 25:585-587; 1998.

C.J. Mathias, S. Wang, P.S. Low, D.J. Waters, and M.A. Green. Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts, *Nucl. Med. Biol.*, 26:23-25; 1999.

C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, and M.A. Green. Evaluation of ^{111}In-DTPA-Folate as a Potential Folate-Receptor-Targeted Radiopharmaceutical, *J. Nucl. Med.*, 39:1579-1585; 1998.

S. Wang, J. Luo, D.A. Lantrip, D.J. Waters, C.J. Mathias, M.A. Green, P.L. Fuchs, and P.S. Low. Design and Synthesis of ^{111}In-DTPA-Folate for Use as a Tumor-Targeted Radiopharmaceutical, *Bioconj. Chem.*, 8:673-679; 1997.

C.J. Mathias, S. Wang, R.J. Lee, D.J. Waters, P.S. Low, and M.A. Green. Tumor-Selective Radiopharmaceutical Targeting via Receptor-mediated Endocytosis: Evaluation of a Gallium-67 Labeled Folate-Deferoxamine Conjugate. *J. Nucl. Med.*, 37:1003-1008; 1996.

Martin J. O'Donnell, Biographical Sketch
November, 2000

Educational Training:

1964-1968	**B.S. in Chemistry**, University of Iowa.
1968-1973	**Ph.D. in Organic Chemistry**, Yale University.
1973-1975	**Postdoctoral**, Université Catholique de Louvain, Belgium.

Professional Experience:

1968-1973	**Graduate Student**, Yale University, New Haven, CT with Prof. K.B. Wiberg.
1973-1975	**Postdoctoral Fellow**, Université Catholique de Louvain, Belgium with Prof. L. Ghosez.
1975-1979	**Assistant Professor of Chemistry**, Indiana University-Purdue University at Indianapolis, Indianapolis, IN.
1979-1984	**Associate Professor of Chemistry**, Indiana University-Purdue University at Indianapolis, Indianapolis, IN.
1984-	**Professor of Chemistry**, Indiana University-Purdue University at Indianapolis, Indianapolis, IN.
7-12/1985	**Visiting Professor of Chemistry**, Imperial College of Science and Technology, London, England.

Honors and Awards:

1992	**NCU Fellowship for Research in Japan.**
1995	**1995 Chancellor's Award for Teaching at IUPUI.** This award, the highest campus honor given for teaching at IUPUI, is given annually to a single faculty member in the university.
1996	**1996 President's Award for Distinguished Teaching**, March, 1996. One of five awardees for the entire Indiana University System (eight campuses).

Selected Bibliography:

45. M. J. O'Donnell, S. Wu, I Esikova and A. Mi, "Catalytic Enantioselective Synthesis of Alpha-Amino Acid Derivatives by Phase-Transfer Catalysis, U.S. Patent 5,554,753, September 10, 1996.

50. M. J. O'Donnell, C. Zhou and W. L. Scott, "Solid-Phase Unnatural Peptide Synthesis (UPS)," *J. Am. Chem. Soc.*, 118, 6070-6071, 1996 (see *Chemical & Engineering News*, July 8, 1996, page 32 for a press release about this research).

52. M. J. O'Donnell, N. Chen, C. Zhou, A. Murray, C. P. Kubiak, F. Yang and G. G. Stanley, "Efficient Catalytic Enantioselective Reaction of a Glycine Cation Equivalent with Malonate Anions via Palladium Catalysis," *J. Org. Chem.*, 62, 3962-3975, 1997.

57. M. J. O'Donnell, F. Delgado, C. Hostettler and R. Schwesinger, "An Efficient Homogeneous Catalytic Enantioselective Synthesis of α-Amino Acid Derivatives," *Tetrahedron Lett.*, 39, 8775-8778, 1998.

58. M. J. O'Donnell, F. Delgado and R. S. Pottorf, "Enantioselective Solid-Phase Synthesis of α-Amino Acid Derivatives," Symposium-in-Print on Phase-Transfer Catalysis, T. Shioiri, Ed., *Tetrahedron*, 55, 6347-6362, 1999.

59. M. J. O'Donnell, F. Delgado, M. D. Drew, R. S. Pottorf, C. zhou and W. L. Scott, "Solid-Phase Synthesis of Unnatural α-Amino Acid Derivatives Using a Resin-Bound Glycine Cation Equivalent," *Tetrahedron Lett.*, 40, 5831-5835, 1999.

60. M. J. O'Donnell, M. D. Drew, R. S. Pottorf and W. L. Scott, "UPS on Weinreb Resin: A Facile Solid-Phase Route to Aldehyde and Ketone Derivatives of 'Unnatural' Amino Acids and Peptides," *J. Comb. Chem.*, 2, 172-181, 2000.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Donald L. Durden, M.D., Ph.D.	Associate Professor of Pediatrics & Biochemistry

EDUCATION

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of South Florida, Tampa, FL	B.S.	1977	Microbiology/Zoology
University of Miami School of Medicine, Miami, FL	Ph.D.	1983	Microbiology/Immunology
University of Miami School of Medicine, Miami, FL	M.D.	1985	Medical Doctor
Childrens Hospital of Medical Center, Seattle, WA	Fellow	1987-1988	Pediatric Hem/Onc
Fred Hutchinson Cancer Research Center, Seattle, WA	Fellow	1988-1992	Molecular/Cell Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

Professional Experience:

1999-Present	Associate Professor, Pediatrics, Biochemistry and Molecular Biology, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana.
1993-Apr. 1999	Assistant Professor, Division of Hematology-Oncology, Department of Pediatrics, Childrens Hospital Los Angeles/University of Southern California School of Medicine, Los Angeles, California.
1989-1992	Postdoctoral fellowship, Fred Hutchinson Cancer Research Center, Seattle, WA, Role of tyrosine phosphorylation in myeloid signal transduction, Jonathan Cooper, Supervisor.
1979-1985	Graduate/Medical Student Research, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL. Isolation and characterization of Vibrio L-asparaginase. J.A. Distasio, Advisor.

Clinical Experience:

1993-1999	Attending Neurooncologist, Division of Hematology-Oncology, Childrens Hospital Los Angeles, LA CA
1999-present	Attending Neurooncologist, Division of Hematology-Oncology, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN.

SELECTED PUBLICATIONS:

1 Wen, S., Stolarov, J., Su, J.D., Donner, D.B, Mayo, L.D., Wigler, M.H., Tonks, N.K., **Durden, D.L.** PTEN controls the tumor induced angiogenic response. *Nat. Medicine.* Submitted. 2000.

2 Erdreich-Epstein, A., Shimada, H., Groshen, S., Liu, M., Metelitsa, L., Kim, K.S., Stins, M., Seeger, R.C. and **Durden, D.L.** Integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ are expressed by endothelium of high-risk neuroblastoma and their inhibition is associated with increased endogenous ceramide. *Cancer Research*, 60:712-721, 2000.

3 Park, R.K., Erdreich-Epstein, A., Liu, M., Izadi, K.D., and **Durden, D.L.** High affinity IgG receptor activation of Src family kinases is required for modulation of the Shc-Grb2-Sos complex and the downstream activation of the nicotinamide adenine dinucleotide phosphate (reduced) oxidase. *J. Immunol*, 163:6023-6034, 1999.

4 Park, Rae-Kil, Izadi, K., Deo, Y.M., Liu, Y.B. and **Durden, D.L.** Role of Src in the modulation of multiple adaptor proteins in FcαRI oxidant signaling. *Blood*, 94:2112-2120, 1999.

5 Erdreich-Epstein, A., Liu, M. Kant, A., Izadi, K., Nolta, J. and **Durden, D.L.** CBL functions downstream of Src kinases in FcγRI signaling in primary human macrophages *J. Leuk. Biol*, 65:523-534, 1999.

6 Izadi, K., Erdreich-Epstein, A., Liu, Y., and **Durden, D.L.** Characterization of Cbl-Nck and Nck-Pak1 interactions in myeloid FcγRII signaling. *Exp Cell Res*, 245:330-342, 1998.

7 Kyono, W. T., De Jong, R., Park, R. K., Liu, Y.B., Heisterkamp, N., Groffen, J. and **Durden, D.L.** Differential interaction of Crkl with Cbl or C3G, Hef-1 and γ-subunit ITAM in myeloid FcγRI signaling. *J. Immunol*, 161:5555-5563, 1998.

8 Chu, J., Liu, Y, Koretzky, G.A. and **Durden, D.L.** SLP-76-CBL-Grb2-Shc interactions in FcγRI signaling. *Blood*, 92:1697-1706, 1998.

9 Park, R.K., Liu, Y.B., Kyono, W., and **Durden, D.L.** CBL-GRB2 adapter protein interaction in immunoreceptor tyrosine activation motif (ITAM) signaling. *J. Immunol*, 160:5018-5027, 1998.

10 Epstein, A., Liu, M., Liu, Y.B. and **Durden, D.L.**, Protein tyrosine phosphtase inhibitors in FcγRI induced myeloid oxidant signal transducton. *Exp. Cell Res*, 237:288-295, 1997.

11 Taylor, N., Jahn, T., Smith, S., Uribe, L., Liu, Y.B., **Durden, D.L.**, and Weinberg, K. Differential activation of the tyrosine kinases ZAP-70 and SYK in FcγRI signaling. *Blood*, 89:388-396, 1997.

12 Park, R.K., Liu, Y.B., and **Durden, D.L.** A role for Shc, Grb2 and Raf-1 in FcγRI signal relay. *J Biol Chem*, 271:13342-13348, 1996.

13 Arditi, M., Zhou, J., Martine, T., **Durden, D.L.**, Stins, M., and Kim, K-S. Lipopolysaccharide stimulates the tyrosine phosphorylation of mitogen-activated protein kinases, p44, p42, and p38 in vascular endothelial cells in a soluble CD14-dependent manner. Role of protein tyrosine phosphorylation in lipopolysaccharide-induced stimulation of endothelial cells. *J Immun*, 155(8):3994-4003, 1995.

14 **Durden, D.L.**, Kim, H.M., Calore, B., and Liu, Y.B. The FcγRI receptor signals through the activation of *hck* and MAP kinase. *J Immun*, 154:4039-4047, 1995.

RESEARCH PLAN

A SPECIFIC AIMS

The proposed research has the following specific aims:

1) Develop and communicate new solid-phase synthetic methodology for macrocyclic chelating agents. MILESTONE: successful library production (>1000 members), at least 2 publications.

2) Preparation of α_vB_3 integrin antagonists based around conformationally restricted chelating agents complexed with therapeutic radioactive metal ions. MILESTONE: *in vivo* tumor uptake (>4% injected dose per gram at 2 hour) and retention of radiolabeled constructs in the tumor vasculature (>2% injected dose per gram at 24 hours)

3) Design and construct multivalent α_vB_3 integrin receptor binding molecules possessing superior retention at the target site (tumor neovasculature). MILESTONE: successful synthesis of multivalent construct having 10X higher in vitro binding affinity and 2X in vitro tumor localization when compared to univalent versions.

This proposal represents an opportunity for experts in several disciplines (chelating agents, vascular biology, combinatorial chemistry, nuclear medicine, medicinal chemistry) to come together to capitalize on tumor vasculature targeting strategies to selectively deliver therapeutic radioisotopes to α_vB_3 integrin-postitive tumors. This is to be accomplished using a novel and genreral approach mimicking antibody-type interaction via spatial arrangements of recognition units using conformationally restricted metal-ligand complexes as scaffolds.

B SIGNIFICANCE

Background and Existing Knowledge

Cancer research has been increasingly focused on tumor vasculature as a potential target for new therapies. Agents such as angiostatin and endostatin have been discovered which can potentially prevent the formation of new blood vessels (angiogenesis) and thus prevent further growth of solid tumors[1,2].

More recently another approach has been described which seeks to take advantage of the differences between normal tissue vasculature and the new vasculature (neovasculature) supporting tumors for the purposes of selectively targeting of drugs to tumors. These differences in vasculture have been noted in the physiology[3] of tumors as well as more recently at the molecular genetic level[4] of endothelium tissue. Monoclonal antibodies (Mabs) that recognize tumor vasculature specific antigens have been labeled with the alpha-emitter isotope ^{213}Bi and found to extend the life-span of tumor laden mice[5]. However, monoclonal antibodies as delivery agents in humans have significant hurdles in becoming therapeutic delivery agents[6]. In particular, Mabs, proteins and large polypeptides suffer from many problems as *in vivo* agents and, in fact, Bristol-Myers Squibb gave up work on angiostatin only last year in favor of developing small molecules that would mimic the effects of the large proteins[7].

Tremendous advances have been made in finding small molecules such as peptides that will target specific receptors *in vivo*. For example Erkii Rusolahti and Renata Pasqualini of the Cancer Research Center at Burnham Institute, La Jolla, Calif., have used phage display peptide libraries to find low molecular weight peptides containing the RDG (Arg-Gly-Asp) sequence that attach selectively to endothelial cells in the vasculature of tumors 40-80 times higher than to endothelial cells in other tissues[8]. The tumor associated receptors for these peptides appear to be the $\alpha_v\beta_3$ integrins which are receptors for vascular growth factors[9]. The $\alpha_v\beta_3$ receptor is widely reported to be highly expressed on many tumor cells (osteosarcomas, neuoroblastomas, glioblastomas, melanomas, and carcinomas–lung, breast, prostate, and bladder)[25]. The number of receptors per cell, an important consideration in targeting therapies where quantities of drug delivered are important, has been estimated to be up to 125,000 per expressing endothelial cell[25]. However, it should be noted that while $\alpha_v\beta_3$ integrin is selectively expressed in angiogenic blood vessels versus normal endothelial cells there are other sites *in vivo* that also express this receptor under normal conditions (notably osteoclasts[26]). The RGD-containing peptide sequences isolated by Rusolahti, possesing high binding selectivity for the $\alpha_v\beta_3$ integrin receptor have been tagged with anticancer drugs such as doxorubicin[8,10] and shown to enhance the efficacy of the drug against human breast cancer xenografts in nude mice versus the unmodified doxorubicin control. This was the first

example of using the selective localization of a low molecular weight ligand binding to tumor vasculature-associated $\alpha_v\beta_3$ integrin to deliver a therapeutic anticancer drug.

The use of the peptide approach to bind with $\alpha_v\beta_3$ integrin receptors exploiting radionuclides as the toxiphore, targeting the neovasculature of tumors, has been proposed[11] but only limited work has been published[19,20]. The most detailed study examined several radioiodinated cyclic RDG peptides which were modeled after the previously optimized cyclo-(-Arg-Gly-Asp-D-Phe-Val-) pentapeptide system. For this cyclo-pentapeptide series they found that a hydrophobic amino acid in position 4 (D-Phe substitution) increases the receptor affinity whereas the position 5 (valine substitution) had little influence on the affinity. This series of cyclo-pentapeptides (including the iodinated tryrosine replacement for D-Phe analog called P2) were shown to be nanomolar inhibitors of the vitronectin receptor $\alpha_v\beta_3$ integrin. Moreover, they were selective for the $\alpha_v\beta_3$ integrin receptor over the $\alpha_{IIb}\beta_3$ receptor which is a glycoprotein involved in platelet aggregation. In order to avoid side effects that would be anticipated by affecting the platelet aggregation process it is critical that the affinity for the widespread $\alpha_{IIb}\beta_3$ integrin receptor is very minimal. Thus, all studies on $\alpha_v\beta_3$ integrin binding need to include a comparison binding study with $\alpha_{IIb}\beta_3$ integrin to evaluate this important parameter. The biodistribution data of the analog radioiodinated $\alpha_v\beta_3$ integrin binding peptide P2 is shown in the Table 1 below. Good initial localization in the tumors is noted but very quick clearance over a short 4 hour time period occurs[19]. The blood component clears even more quickly resulting in increasing tumor/blood ratios from 10 minutes to one hour time but essentially remaining constant through the four hour time period. The thyroid accumulates considerable isotope which is probably due to *in vivo* deiodination. Lastly, there is significant liver localization early on diminishing over time consistent with hepatobiliary clearance of the peptide. The loss of activity from the tumor site is not discussed by the authors but could be due to the lack of internalization of the antagonist at the receptor site. These results indicate that from a therapeutic standpoint there remains some optimization to be performed on this cyclo-pentapeptide system.

Table 1. Evaluation of radioiodinated tyrosine-containing cyclo-pentapeptide P2 [cyclo-(-Arg-Gly-Asp-D-Tyr-Val-)] in mice bearing tumors19 shown as % Injected Dose/gram

Tissue	Melanoma M21			Osteosarcoma			Mammary Carcinoma		
	10 min	60 min	240 min	10 min	60 min	240 min	10 min	60 min	240 min
Tumor	2.07	1.30	0.41	3.50	1.46	0.92	1.84	0.74	0.72
Blood	0.77	0.17	0.06	1.72	0.17	0.12	0.73	0.10	0.09
Muscle	0.42	0.25	0.10	0.94	0.36	0.24	0.48	0.16	0.14
Liver	21.96	11.23	0.78	19.06	4.22	2.18	25	12	1.33
Thyroid	2.21	3.45	0.3	3.49	15.61	30.02	5.40	1.88	4.90
Tumor/Blood Ratio	2.7	7.7	6.8	2.0	8.6	7.7	2.5	7.4	8.0

Habner and coworkers have extended the use of this cyclic pentapeptide, as described in recent presentations, by attaching the radioisotopes F-18, ^{188}Re, ^{90}Y and ^{99m}Tc to closely related derivatives of c(RGDfV) wherein the V (valine) has been replace by K (lysine) covalently modified on the epsilon-amino group[23,24] to contain a moiety capable of binding the radioisotope. The published data[23,24] showed a similar pattern of diminished absolute amount of isotope located at the tumor over time after initial uptake but accompanied by increasing tumor-to-blood ratios. This is the same pattern noted in Table 1 indicating that the loss of tumor associated activity over time is not due to the inherent biological clearance problems associated with iodinated biomolecules but must be due to a pharmacokinetic process.

The appeal of employing a radionuclide in this approach, targeting neovasculartune of tumors, is that no drug has to be liberated to perform the therapy and the radiation could be effective in either destroying the tumor-supplying blood vessels or directly destroying the tumor cells themselves since the site of the neovasculature localization is in such intimate proximity to the tumor cells in small metastatic lesions. Ideally, the radiation

selectively localized to the neovasculature of metastatic tumors could work via both of these mechanisms if the proper radioisotope is utilized. For example, the penetration distance for the maximum energy particle (β^-) emitted for $^{153}Sm+3$ is estimated at only 3.4 mm versus 8.6 mm for $^{166}Ho+3$. Thus, the choice of isotope should be matched to the phamacokinetics of the delivery agent as well as the size of tumor being treated. The potential value of just targeting the destruction of the neovasculature alone should not be underestimated as it has been estimated [11] that 100 tumor cells die for each destroyed endothelial cell in tumor blood vessels illustrating a possible amplification of the therapeutic localization of radioisotopes in tumor neovasculature.

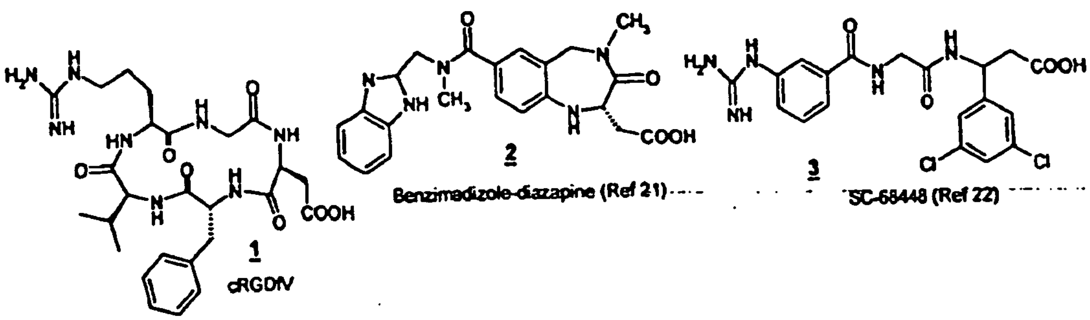
One drawback or disadvantage to using radioiodinated peptides such as the vascular targeting agents described above in Table 1 to selectively target tumors is their susceptibility to natural levels of peptidases and proteases which leads to extremely fast clearance rates from the bloodstream. While this may sometimes be useful for imaging purposes to yield a better target-to-nontarget ratio it is unacceptable in a therapeutic approach as it lowers the absolute amount of drug reaching the target[12]. Additional problems exist with radioiodinated peptides as opposed to chelated-metal-labeled peptides and that is the radioiodinated peptides are converted to iodotyrosines and iodide both of which clear quickly from the targeted site making the agent unacceptable in a therapeutic setting[12]. The obvious remedy of using a bifunctional chelating agent to attach radiometal ions to peptides, as an alternative to radioiodination, also presents problems in that because of the low molecular weight of the peptides (versus monoclonal antibodies) the presence of the attached metal complex can dramatically affect the biodistribution and pharmacokinetics of the low molecular weight radiolabeled peptide. In fact, a recent review stated that various studies have demonstrated "the essential role that the chelation and conjugation chemistries play in determining the *in vivo* uptake and phamacokinetic behavior of radiolabeled receptor-avid peptides being designed as potential therapeutic radiopharmaceuticals"[13]. Thus, **a peptide that has been optimized for targeting a receptor is likely to be suboptimized when a chelated metal ion is then conjugated to it.** This can be attributed to the addition of significant molecular weight as well as significant changes to the lipophilicity, molecular electronics, and steric environment of the ligand with regard to specific receptor binding interaction.

Investigators have studied the use of peptidomimetics to overcome the peptide limitations described above (fast clearance, metabolization) with some notable successes. For example, β-peptides have been used with success to mimic peptides as demonstrated by a cyclic β-tetrapeptide as a mimetic of somatostatin[14]. A more dramatic example is the use of nonpeptide-like templates used to present mimetics of individual key binding residues of peptides in their interactions with a receptor. The cyclic peptide bioactive somatostatin is represented in binding by a very different-looking mimetic based on β-D-glucose[15,16]. Binding assay results support the hypothesis that the glucose template (scaffold)-based presentation of binding groups can mimic somatostatin's biological activity.

This same approach did not work as well in the area of designing peptidomimetics for the $\alpha_v\beta_3$ antagonist cyclo(-Arg-Gly-Asp-D-Phe-Val-) [abbreviated as cRGDfV, 1] based on a carbohydrate template. In this work of Nicolaou et al. they first determined the solution structure of cRGDfV by NMR[17]. Based on molecular modeling Nicolaou proposed and synthesized a handful of cRGDfV analogs based on the pyranose carbohydrate ring system as a template. Unfortunately, little to no binding of these mimics to α_vB_3 integrin was observed. The authors suggest that there may exist subtle requirements for the active cyclic peptide conformation which may not be fulfilled by these mimics as well as perhaps a lack of sufficient rigidity associated with the carbohydrate framework[17].

Others have been more successful in finding peptidomimetics of cRGDfV (1) based on other templates. Benzodiazapines such as structure 2 have been found to be low-nanomolar inhibitors of vitronectin binding to α_vB_3 integrin with a 10000-fold selectivity over undesirable inhibition of $\alpha_{IIb}\beta_3$ receptor[21]. In this case the 1,4-benzodiazepine acts as a Gly-Asp mimic with the benzimidazole unit acting as an arginine mimic. Another RGD peptidomimetic selective inhibitor of α_vB_3 integrin was identified[3] (3, SC-68448) which showed up to 80% reduction in tumor growth in a mouse-based Leydig cell tumor model[22]. This molecule is simply an open chain analog presenting a guanidine moiety (arginine mimic) and a carboxylic acid (aspartic acid mimic) separated by a spacer group which allow for their presentation in a spatial arrangement that recognizes the α_vB_3 integrin

Figure 1. Structure of c(RDGfV) and nonpeptide mimetics.



1 cRGDfV — 2 Benzimadizole-diazapine (Ref 21) — 3 SC-68448 (Ref 22)

cyclo-(-Arg-Gly-Asp-D-Phe-Val-)

receptor. It should be noted that 2 and 3 are not disclosed as targeting agents but are examples of cRGDfV peptidomimetics that are selective $\alpha_v B_3$ integrin receptor antagonists (selective relative to the $\alpha_{IIb}\beta_3$ receptor).

Commercial Opportunities

ComChem Technologies Inc. (CCTI) is a start-up company formed to discover and commercialize diagnostic and therapeutic radiopharmaceuticals. CCTI's strategy is to utilize combinatorial chemistry in conjunction with chelating agent expertise to explore new areas and to arrive at commercializable products quicker than its competition. This requires close collaboration with others possessing complementary expertise such as radiochemistry, medicine, and biochemistry.

CCTI has a competitive advantage in that the PI of this research proposal has a proven track record in inventing, developing, and bringing therapeutic radiopharmaceuticals into human clincal trials. He was instrumental in the development and first human trials of FDA approved Quadramet (licensed by Dow to Cytogen) as well as lead inventor and project champion for all aspects of ^{166}Ho-DOTMP which has now progressed to phase III human clinical trials (STR licensed by Dow to NeoRx Corporation).

The technology that will be developed in this proposal has a specific commercial application but also has broad application as a new method to produce three-dimensional presentation of molecular recognition units in a compact molecular space that is ideal for radiotherapy. The intellectual property expected to be generated herein will be protected by filing US and overseas patent applications.

Importance of Proposed Research

This Phase I work will lay the foundation for preclinical and clinical evaluation of tumor vasculature localizing radiotherapy for cancer treatment in Phase II. This agent will be broadly applicable to treating all $\alpha_v B_3$ integrin-positive solid tumors with targeted radiotherapy. It has taken over 15 years for a monoclonal antibody (Rituxan) to finally achieve FDA approval for treating lymphoma. A radiolabeled version recently finished phase III trials and has been submitted to the FDA for approval. We believe the use of combinatorial chemistry applied to the problem of finding an optimum radiolabeled low molecular weight vascular localizing agent will allow for much faster discovery and development timelines. The commercial potential of this approach is enormous and the cost-of-goods expected to be much lower than an antibody approach which should result in a lower cost of the drug from the patient's perspective.

C **RELEVANT EXPERIENCE. Principal Investigator;** Dr. Garlich, CCTI Chief Scientist, has eleven years of industrial experience at Dow Chemical in the area of radiopharmaceutical discovery and development. He was instumental in the synthesis and formulation development for ^{153}Sm-EDTMP, an FDA approved radioactive drug for the relief of bone pain associated with bone metastases, licensed to Cytogen Corp.(Quadramet™). He also developed new azamacrocycles (synthesis and new uses) as well as bifunctional chelating agents for monoclonal antibodies. He is the father of ^{166}HoDOTMP, a bone-seeking radiopharmaceutical, now in phase III clinical trials for the treatment of multiple myeloma (licensed by Dow to NeoRX). More recently, he was responsible for establishing the combinatorial chemistry group at Dow

AgroSciences and has experience in all aspects of combinatorial chemistry-automation, solid-phase and solution phase synthesis, analytical instruments and methodology.

Co-Investigator; Professor Mark A. Green has a background in inorganic chemistry and 18 years of productive research experience in the design, synthesis, and evaluation of new metal-based radiopharmaceuticals. His group is internationally recognized for their efforts in development and pre-clinical testing of low-molecular-weight copper radiopharmaceuticals for imaging with positron emission tomography. For tumor imaging, his group has also pioneered efforts in tumor targeting with low molecular weight folate-chelate conjugates that target a tumor-cell-membrane-associated receptor for folic acid. In addition, they have developed and evaluated an extensive series of monocationic gallium radiopharmaceuticals that are substrates for transport by the MDR1 P-glycoprotein involved in tumor multidrug resistance.

Project Coordinator; Carla J. Mathias brings a background in zoology and chemistry to this project, along with 21 years experience in the design, synthesis, pre-clinical testing, and clinical evaluation of new radiopharmaceuticals. She is experienced in techniques of radiochemical synthesis and analysis, as well as the development and application of animal models for assessment of new radiopharmaceuticals. Her experience includes synthetic, animal, and human studies related to the evaluation of radiolabeled platelets and white cells, radiolabeled antibodies, 18F-labeled estrogen receptor ligands for imaging breast tumors with PET, generator-based PET perfusion tracers, and low molecular weight radiopharmaceuticals targeted to tumor-associated receptor systems.

Consultants; Dr. O'Donnell pioneered the area of unnatural peptide synthesis which serve as key intermediates in the synthetic aims of this proposal. His interaction will be extremely valuable in achieving the synthetic goals. Dr. Durden, MD, Ph.D. has extensive experience and expertise in vascular biology and integrins. He is an expert in signaling transduction and has much valuable experience in biochemical assays in this area.

D RESEARCH PLAN:

Experimental Plan Stage A & B Rationale and Introduction

Given the drawbacks and approaches described above in the Background section it would be desirable to treat cancers that are highly expressing α_vB_3 integrin by a small nonpeptide molecule that 1) possesses a built-in chelating agent complexed with a therapeutic radioactive metal ion in a stable fashion and 2) the resulting nonpeptide metal-ligand molecule possesses a high affinity and selectivity to the α_vB_3 integrin. We propose to achieve this with conservation of atoms by using the chelating agent moiety itself as the template upon which to place the α_vB_3 integrin binding moieties in a spatial arrangement that mimics the well known α_vB_3 integrin antagonist c(RDGfV). The synthesis involved in this approach is detailed in Stage A below. Expanding on this approach is our proposed design to use the chelating agent as the platform from which to tether multiple copies of a selective α_vB_3 integrin-binding moiety such as c(RDGfV). This multivalent approach (Stage B), a relatively new concept and not yet applied to integrin binders, will be approached combinatorially to find the optimum distances between the multiple copies of the binding moiety and to study the effect of different spacing groups on the binding of the resulting construct with integrins. The astute reader will recognize after examining the generic schemes that there is some crossover from Stage B into Stage A in that some of the members of Stage A can contain multiple copies of presented binding moieties. This is not an intent to confuse the reader but reflects the great flexibility built into the synthetic approaches.

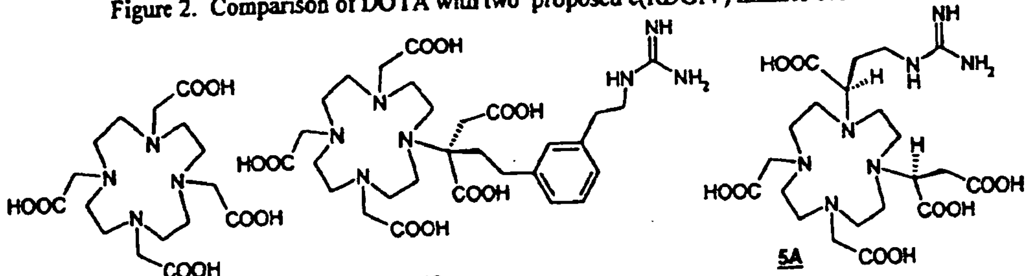
Synthesized molecules that mimic the binding of monoclonal antibodies are called chemobodies[35]. We have coined the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding. Compounds described in both Stage A and Stage B fit into this new category of chelabodies.

Research Plan Stage A: Preparation of RDG Mimics Based Upon Macrocyclic Complexes (Chelabodies)

The chelating agent DOTA, 4 (1,4,7,-10-tetraazacyclododecane-tetraacetic acid), is well know to form kinetically inert complexes with the lanthanides[28] and the resulting complexes are considered conformationally ridgid[29]. The resulting complexes are overall negatively charged at physiological pH when complexed with a trivalent metal ion. The attractiveness of a complex utilizing lanthanides as the metal ion is attributable to the variety of radioactive lanthanides in use in nuclear medicine ($^{153}Sm^{+3}$, $^{90}Y^{+3}$, $^{166}Ho^{+3}$) with differing half-lives and beta-particle energies. The lanthanides tend to be quite similar in their complexation chemistry so that the design of one system may allow the use of any one of several therapeutic radioactive lanthanide metal ions (ie thus more flexibility in choosing the proper radioisotope based upon biological half-life). It should be noted that the Principal Investigator has extensive experience (synthesis, complexation, and radiochemistry expertise) with lanthanides and macrocyclic chelating systems that has led to one commercial drug (Quadramet) and one drug in Phase III clincal trials (STR being evaluated by NeoRx Corporation). Another attractive feature of the DOTA chelator system is its widespread use in clinical MRI imaging agents and bifunctional chelating agents for attaching radioactive lanthanides to monoclonal antibodies for use in humans.

An inspection of molecular models of DOTA complexes indicates that DOTA is similar in size to the peptide ring α_vB_3 integrin antagonist c(RDGfV). This led us to the idea that suitable c(RDGfV) mimics could be prepared by judicious subsititution patterns on the DOTA backbone. For example, molecular modeling indicates that structure 5 (DOTA-RXG)when complexed with Y^{+3} would place the guanidine and carboxylic acid in a similar spatial arrangement as that found for the guanidine of the arginine and the carboxylate of the aspartic acid residues in c(RDGfV)[29]. Likewise, from modeling estimates structure 5A (upon complexation with Y^{+3}) appears to also satisfy the spatial requirements of the binding moieties of c(RDGfV)[29]. Stucture 5 represents a single arm attachment and structure 5A represents adjacent chelating arm modifications. It should be noted that modeling indicates that similar achievement of a c(RDGfV mimic using modifications of acetate arms that are not adjacent would be difficult unless extremely large and conformationally floppy spacer groups are used. Thus our effort will be focused initially on 5 and 5A and their analogs.

Figure 2. Comparison of DOTA with two proposed c(RDGfV) mimics based on DOTA modifications.



DOTA   DOTA-RXG

4   5

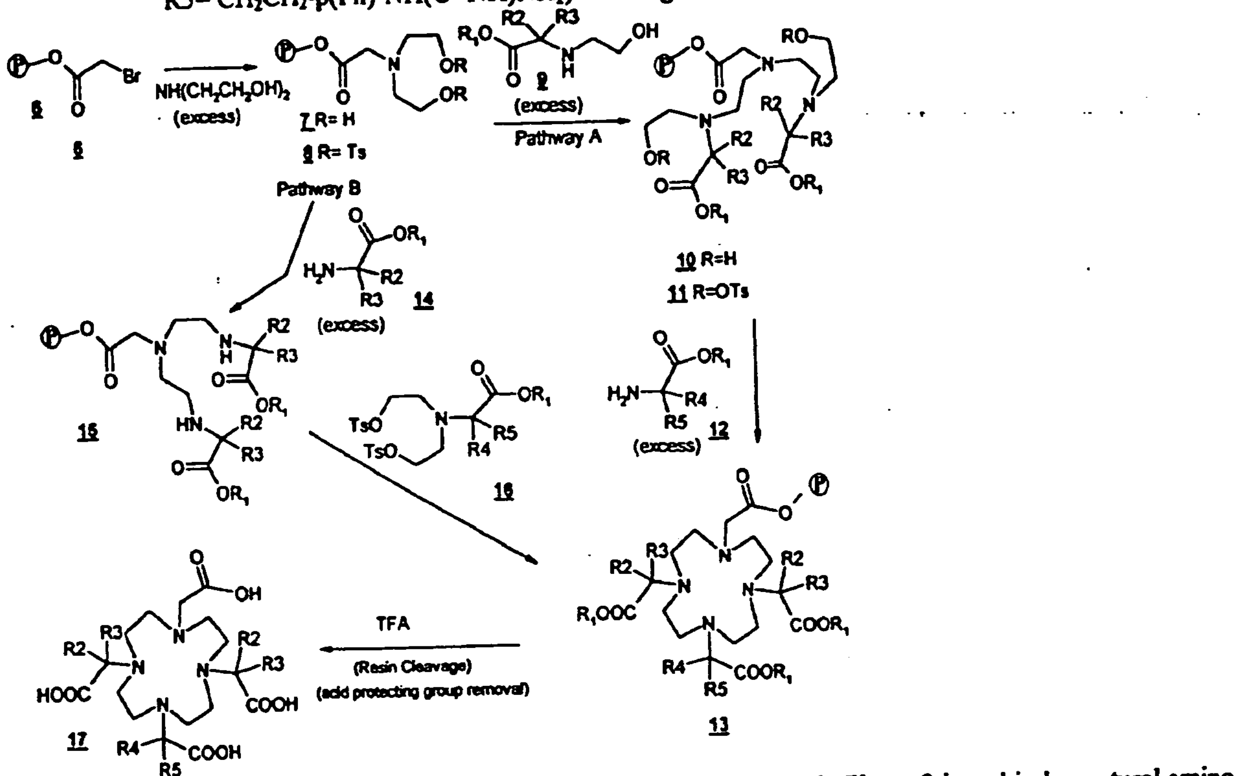
There are numerous other possible substitutions on the acetate arm besides those shown in 5 and 5A which could restrict rotation even further to provide additional preorganization to mimic c(RDGfV). Additionally there are many additional groups that can serve as carboxylate mimics and guanidine mimics. Our plan is to prepare a library of compounds similar to 5, guided by molecular modeling, via the solid-phase combinatorial chemistry route proposed in Figure 3.

In Figure 3 the circled P represents the solid phase resin, Wang resin in this case. However, the use of Rink amide resin is also to be evaluated which would give a DOTA-based chelator wherein one of the chelating acetate arms is a -CH2C(O)NH2 group upon cleavage from the resin. These types of chelators are known and while they are not as stable as DOTA they are stable enough for *in vivo* use[29]. An additional advantage of this monoamide from Rink amide resin would be that the resulting complex with trivalent lanthanides would give a neutral complex core molecule. This could have important *in vivo* biodistribution effects which will be studied.

The synthetic scheme (Figure 3) to prepare these molecules illustrates two pathways to get to the same desired substituted DOTA chelator, 17. Both pathways will be examined and each will require significant

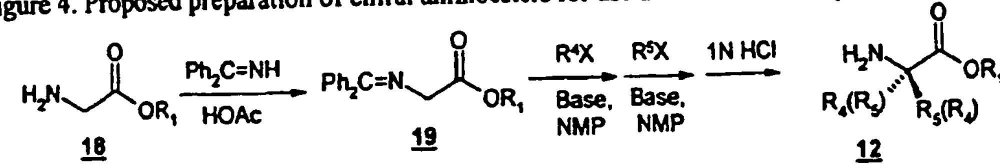
* optimization work. These efforts would represent the first on-resin synthesis of the medically important tetraazacyclododecane ring system. We thus feel that this work, even if ultimately unsuccessful in the biological evaluation, will be a welcome and exciting combinatorial chemistry methodology advance in the area of chelation based inorganic medicinal chemistry. By using R2=R3=H the synthesis as shown in Figure 3 simplifies to only one chelator arm substituted with two moieties. The stereochemistry is not shown in Figure 3 but the use of the proper enantiomer of 12, which we plan to isolate and obtain in each instance, will deliver the desired stereoisomer as shown in structure 5.

Figure 3. Proposed solid-phase synthesis of 5 (R2=R3=H; R4=CH_2COOH; R5= CH_2CH_2-p(Ph)-NH(C=NH)NH_2) as a single member of a combinatorial library.



* The key building unit to get to structures like 5 via the route shown in Figure 3 is a chiral unnatural amino acid derivative. A diverse collection of these disubstituted glycine derivatives can be prepared in solution phase or solid phase by the UPS (unnatural peptide synthesis) route pioneered by O'Donnell who is serving as a consultant on this proposal[31,32]. This procedure is shown in Figure 4 and lends itself to automation[33]. It is anticipated that the different enantiomers resulting in Figure 4 will be separated using chiral chromatography. There are methods to perform the chemistry in Figure 4 wherein either R4 or R5 is hydrogen with significant stereoselectivity (80-90% ee) but our criteria for purity (>95%) requires that we perform a chiral separation at this stage. This will be performed using HPLC methodology.

Figure 4. Proposed preparation of chiral aminoesters for use in combinatorial synthesis(Figure 3).

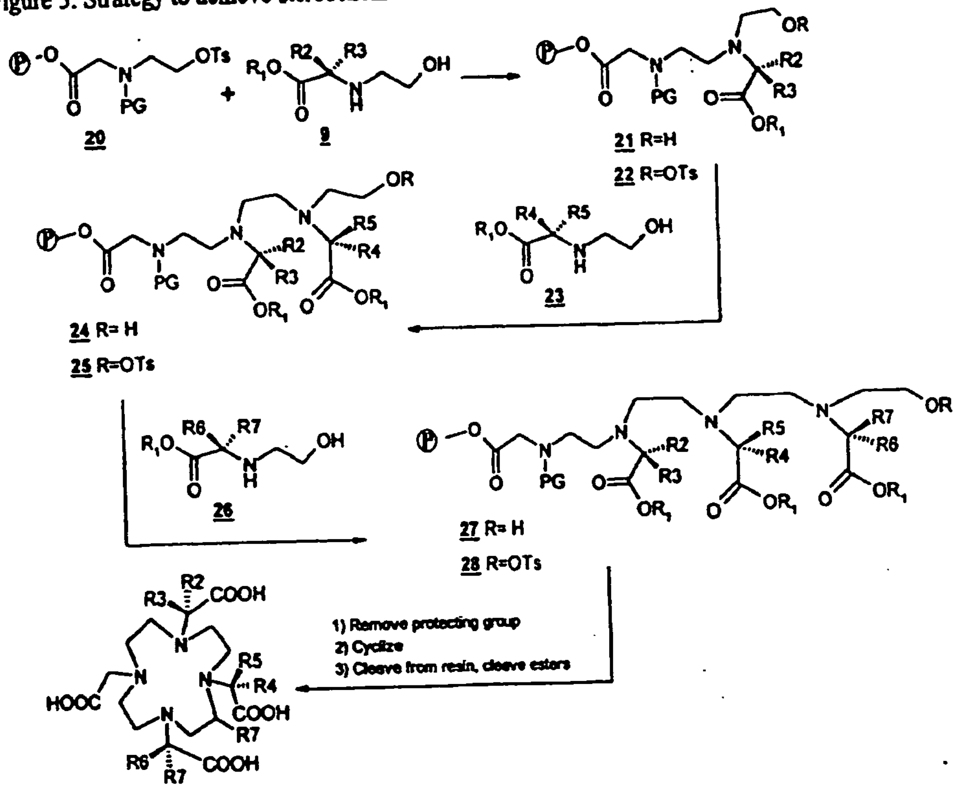


* With the inputs 12 (and 14 which can be the same or different from 12, derived from the same chemistry) in hand then the library production protocol based on structure 5 can be developed. Because of the way the

* synthesis is developed it is possible to make an analog of 5 where each of the three acetate arms contain one copy of the RDG mimic structure by making 12 and 14 the same aminoester. This trivalent species, by benefit of compact presentation of three copies of the RDG mimic strucure, could possess some interesting properties. There is more discussion later regarding this multivalent approach in the research plan stage 2 discussions.

* In order to access desired target molecules such as 5A a different synthesis route is needed since two identical molecules of aminoester are incorporated in either pathway A or pathway B in Figure 3. This uncontrollable dual incorporation precludes introducing the needed stereochemistry at both sites, i.e. only one acetate substitution pattern will have the correct configuration. To address the desired access to molecules like 5A and to give complete control over the stereochemistry of all 6 substituents on the chelating acetate arms the synthetic protocol shown in Figure 5 will be evaluated. The amino alcohols 9, 23, and 26 will be prepared from the corresponding unnatural amino esters prepared by the method shown in Figure 2 and purified to get the single isomer. The preparation of these aminoalcohols could make use of resin bound ethylene glycol wherein the amine of the amino ester (such as 12) displaces the activated non-resin bound hydroxyl of the ethylene glycol. The PG (protecting group) on the nitrogen of Figure 5 will be determined after some preliminary work is

Figure 5. Strategy to achieve stereochemical control at each chiral acetate arm position such as 5A.



* performed to ensure othogonal stability but likely will be a group such as FMOC, NOSYL, or trifluoracetamide.

These proposed chelator scaffolds (chelabodies) addresses all of the shortcomings described previously for a tumor neovasculature seeking agent. The positive attributes for this system are 1) nonpeptide in nature so not prone to metabolism; 2) incorporates a kinetically inert lanthanide complex which allows for a potential range of radioisotopes having varied particle energies and half-lives and yet produced commercially (Sm-153, Ho-166, and Lu-177); 3) rigid backbone (cyclododecane ring system locked into place upon chelation) upon which to place appropriately spaced recognition/binding groups; 4) the complex containing the toxiphore (radioactive metal ion) is part of the core rigidifying structure so no additional conjugation chemistry is required, i.e. the compound from screening will not need to be further modified to label with a radioactive isotope;

Research Plan Stage B:

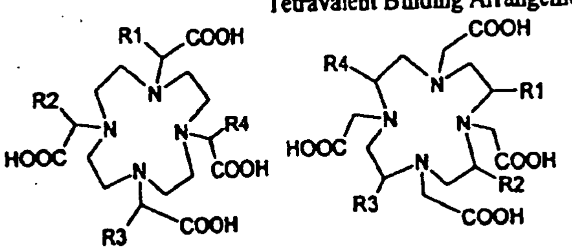
Preparation of Extended Multivalent RDG Mimics Based Upon Macrocyclic Complexes (Chelabodies)

* Monoclonal antibodies are known for their exquisite selectivity and high binding affinity. These attributes arise in part because antibodies are divalent and in some cases multivalent in their binding with proteins or receptor surfaces. Nature has used multivalent binding to overcome weak binder in order to make strong attachments[35]. Multivalency, simultaneous attachment of two or more binding sites on one molecule (drug) to multiple receptor sites on another (cell surface), is a new approach to drug design according to George M. Whitesides of Harvard University[35,36]. This multivalent approach has not yet been applied to ligands aimed at binding the integrins although Burgess has disclosed a cyclic sequence, c(RDGRGD), that could be considered a dimer of RDG[37]. Surprisingly this ligand possessed excellent selectivity and antagonistic activity towards α_vB_3 integrin.

* This area of multivalent drug design is where the term "chemobody" has been coined to describe synthesized molecules that mimic the binding of monoclonal antibodies[35]. We are proposing the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding.

* Research plan stage B comprises the design and evaluation of multivalent presentations of α_vB_3 integrin antagonists based on the DOTA template. This is illustrated conceptually in Figure 6 where either four substitutions are made on the chelating arms (<u>30</u>) or situated around the macrocyclic ring (<u>31</u>). We have also considered the possibility of a mixed species where some substitution is on the acetate arms and some is on the backbone carbons but no compelling reason exists to pursue this approach over the other two described here in more detail. Given the resource available in this proposal we will put our effort in the arm substituted system (<u>30</u>) since that approach takes advantage of the chemistry worked out in research plan A. The focus of this proposal is for the R groups to contain, preferably at their terminus, a moiety that is an α_vB_3 integrin

Figure 6. Conceptual design of Chelabodies Based on DOTA-type Chelating Agents Presenting a Tetravalent Binding Arrangement Aimed at α_vB_3 Integrin Antagonism.

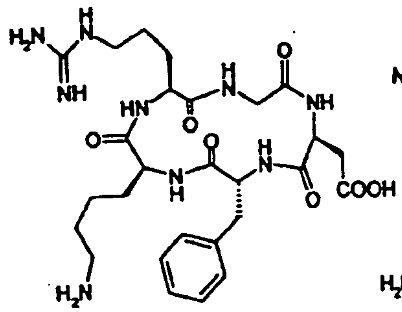


<u>30</u> <u>31</u>

* antagonist. The ideal terminal group would be one that induces internalization of the bound ligand into the cell and compounds will be tested for this property (see biological assay section). In order to prove the concept involved here we first will use known antagonists at the terminal binding positions. For example the known antagonist c(RDGfK) (<u>32</u>) has been described and is amenable to capping off the "R" arms to provide a suitable multivalent antagonist construct. This compound will either be synthesized in-house or custom prepared for CCTI outside of the budget requested here. The linker/spacer arms can be similar to those described in the literature for multivalent constructs, some of which are illustrated in Figure 7. One basic linker arms idea is to react carboxylic anhydrides with a nucleophile such as nitrogen on the arm stub and then couple a diamine with the resulting free carboxylic acid. This procedure is amenable to solid-phase synthesis to prepare arms that are all the same[38,39]. Applying this strategy to the compounds of Figure 4 and Figure 5 requires only that some of the substituents (R2, R3, R4, R5, R6, R7) on the arm building blocks (<u>9</u>, <u>12</u>, <u>14</u>, <u>16</u>, <u>23</u>, <u>26</u>) contain a masked electrophile (to react with amines for example) or nucleophile (to couple with carboxylic acids for example) that

* can be deprotected and then elaborated into a linke/spacer module for endcapping with antagonists such as 32. This approach would work via the chemistry outlined in Figures 4 and 5 to give essentially trivalent constructs (i.e. one per each substituted chelator arm). There is no convenient method to get to a fully symmetrical tetravalent system using solid phase methodology so solution phase methods will be examined. It is apparent that there are a large number of possible constructs that could be prepared varying the nature and length of the arms.

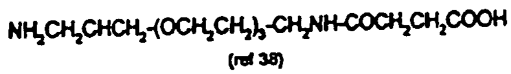
Figure 7. Proposed Endcap Moiety for $\alpha_v B_3$ Integrin Antagonist in a Multivalent Construct and Examples of Linker/spacer Modules.



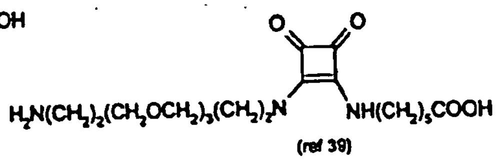
32

c(RDGfK)

cyclo-(-Arg-Gly-Asp-D-Phe-Lys-)



33



34

* Our approach is to prepare a combinatorial library of such constructs and to assess their biological binding and performance (*in vitro* binding and whole cell assays) to determine if improvements in tumor cell localization are possible.

Research Plan Biological Evaluations:

* Assay-In Vitro: The ELISA-type in vitro testing for competitive binding of test ligands with $\alpha_v B_3$ integrin is well established as are the methods to obtain the needed starting materials; vitronectin, $\alpha_v B_3$ integrin, firbrinogen, and $\alpha_{IIb} B_3$ integrin[19, 22, 27, 41, 42, 43]. The procurement of some of these will be at CCIT's cost outside of the budget proposed in this application. Briefly, the solid-phase competitive displacement *in vitro* assay test comprises; 1) coating 96-well plates with $\alpha_v B_3$ integrin receptor (or $\alpha_{IIb} B_3$ integrin receptor to determine selectivity), 2) washing sequence including 1% BSA, 3) exposure to various concentrations of test compound containing biotinylated vitronectin (or biotinylated fibronectin)[19] for 2 hours, 4) washing sequence, and finally 5) detection of biotin present using reporter-labeled anti-biotin antibody. This testing will be performed on nonradioactive metal ion complexed with our newly synthesized compounds so that it can be performed in a medium-throughput mode at the Purdue Center for Combinatorial Chemical Biology.

* Assay- In Vitro Whole Cell Internalization Studies: A recent method has been described to determine internalization of integrins which are thought to occur via endocytosis[44]. Our approach will not necessarily measure internalization (which requires anti-ligand antibodies) but will expose integrin expressing cells to our synthesized ligands and then determine the degree of binding by aggressive exposure to competitive ligand and various washes. Since all of our molecules chelate radioactive metal ions these radioactive metal complexes will be easily determined to be either cell associated, or easily removed. The ultimate location of our ligands is less important than ensuring that the antagonists stay bound to the cell surface so that *in vivo* they are able to deliver the desired radiation dose.
* Animal Studies: *In vivo* evaluation of the best *in vitro* active compounds. The animal testing we will perform will follow those most recently published in the area of nuclear medicine[19]. These animal results using human tumors implanted into immune-compromised mice will provide biolocalization data. We will not be measuring antitumor effects as the animals will be sacrificed to quantitate the tumor and normal tissue uptake. The tumors and cell line we will be using is the melanoma line WM164 available from ATTC.

Specific Goals/Accomplishments Expected for Phase I Year 1:

1 Perform modeling of complexes (chelabodies) that will mimic neovasculature targeting peptide-receptor binding interactions via substitution patterns on a DOTA-lanthanide complex scaffold.
2 Several virtual libraries of complexes are assessed by molecular modeling of receptor fit to determine synthetic direction have been performed.
3 Synthetic methodology has been developed to create macrocyclic chelator based libraries that are mimics for the c(RDGfV) binding ligand.
4 Binding assays are developed to screen libraries, some libraries have been evaluated and some hits are identified. Also, a whole cell binding assay has been evaluated and implemented.
5 Hits from biological screens are confirmed, identified and synthetic effort to optimize at least some of these hits has been initiated.
6 Confirmed hits from biological screens have been evaluated in tumor bearing mice.
7 Work has begun to evaluate the feasibility of making multivalent constructs. Some constructs will have been prepared.

Specific Goals/Accomplishments Expected for Phase I Year 2:

1 Optimized leads from research plan stage A have been evaluated *in vitro* and *in vivo* and are ready for preclincial studies.
2 Synthetic methodology has been developed for preparing multivalent constructs in research plan stage B.
3 Multivalent construct libraries from research plan stage B have been prepared and hits optimized from *in vitro* and *in vivo* testing to give maximum tumor localization of radiometal isotope.

E HUMAN SUBJECTS- NONE

F VERTEBRATE ANIMALS

1. Athymic mice (~135 per year) will be required for screening each new radiopharmaceutical (that shows promise in *in vitro* studies) to determine the agent's tumor localization *in vivo*. We plan to screen and evaluate 15 new radiotracers *in vivo* per year, using nine animals per compound. The tumor-bearing athymic mice are required for assessment of radiotracer distribution and pharmacokinetics, plus demonstrating that the tumor uptake of tracer is mediated by binding to the $\alpha_V\beta_3$ receptor. In this project we will conduct cell culture studies as a preliminary screen of tracer affinity for the $\alpha_V\beta_3$ receptor, to insure that biological data is only collected from animals in cases where there is a good probability of targeting tumor-vasculature-associated receptors, thereby minimizing animal usage as well as experimental expense. The athymic mice will be implanted with human tumor cells (WM164 human melanoma available from ATCC) using standard aseptic techniques, and housed under aseptic conditions until tumor growth is evident. The mice will then be used for biodistribution studies designed to determine the tissue distribution and pharmacokinetics of the test tracers. The radiopharmaceutical will be administered intravenously *via* the exposed femoral vein (to allow visual verification that the dose is completely delivered into the vein) with the animal under diethyl ether anesthesia. Tissues that will be sampled for quantification of radiopharmaceutical uptake include the tumors, blood, heart, lungs, liver, spleen, kidneys, stomach and intestines, muscle, fat, and brain. For each tracer, data will typically be collected at 2 and 24 hours post-injection, examining 3 animals per time point. An additional 3 animals will be examined at one of these time points after co-administration of the radiotracer with an excess of a known high-affinity $\alpha_V\beta_3$ ligand, in order to demonstrate the expected competitive blocking of radiopharmaceutical uptake in tumor. This blocking study will also implicitly provide a measure of the level of non-specific radiotracer uptake in tumor. If it appears likely to assist in interpretation of the resulting mouse data, biodistribution data will also be collected for ^{64}Cu-PTSM and ^{18}F-FDG in the mouse tumor model(s), allowing direct assessment of the rate of tumor perfusion, and rate of metabolism, respectively. The athymic mouse has been chosen as our primary animal tumor model since it can serve as a host for a variety of human tumor cell lines, and is easy to handle and maintain.

2. The use of animal models for screening potential new radiopharmaceuticals is essential to the development of improved diagnostic imaging agents for use in clinical nuclear medicine. The athymic mouse is

the preferred animal for this screening because of their size, low cost, ease of handling, and their ability to act as hosts for human tumors. The number of animals proposed (3 per time point) is the absolute minimum required to obtain statistically reliable data.

3. Veterinary care is available through the Purdue's AAALAC-approved animal facilities. At Purdue University there is one veterinarian and 6 animal caretakers, all assigned full-time to animal care. All of the animal caretakers are certified by AAALAS as ALAT or LAT. Athymic mice will be housed in a specially designed facility within the School of Veterinary Medicine. Dr. David J. Waters, who will be responsible for generation of the mouse xenograft models, is a board-certified veterinary surgeon and is familiar with all aspects of the husbandry and medical care of athymic mice. He is the Director of the Purdue University Athymic Mouse Facility, which provides the required services as a Core Service of the Purdue Cancer Center. Purdue University has on file with OPRR an Assurance of Compliance with Public Health Service Policy on Humane Care and Use of Laboratory Animals (Welfare Assurance #A3231-01). As specified in this policy, all Purdue University programs and facilities for activities involving animals have been evaluated and accredited by the American Association for Accreditation of Laboratory Animal Care. All of the programs and facilities for activities involving animals have also been evaluated by the Purdue Animal Care and Use Committee (PACUC) and are subject to triennial review by PACUC. Professor Green's protocol for the proposed studies is approved by PACUC (PACUC #93-069-99; 10/4/99).

4. All animals will be anesthetized to prevent any pain, distress or discomfort during experimental procedures. For the *in vivo* experiments that require subcutaneous implantation of tumor cells in athymic mice, no surgical procedures are necessary. To minimize discomfort and pain, 25 gauge needles will be used for the implantation of tumor cells. Similarly, only 25 gauge needles, or smaller, will be used for administration of drugs. Treatment of mice will not require analgesics or anesthetics because they require only momentary restraint and minimal discomfort. Dr. Waters is a board-certified veterinary surgeon with extensive experience in administration of methoxyflurane anesthesia and surgical implantation of tumor cells in athymic mice. In our experience, mice recover smoothly from methoxyflurane anesthesia and resume normal activities (e.g., eating, grooming) within a few hours after the procedure. In no cases will the tumors be allowed to grow on animals to the point where their health is compromised. The polycarbonate cages and microisolator system used are approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC) for housing athymic mice. Mice will be anesthetized by inhalation of diethyl ether to produce unconsciousness prior to radiotracer injection and sacrifice.

5. Mice will be sacrificed by decapitation under anesthesia as specified above (rapid sacrifice and excision of organs is required to obtain reliable data in the biodistribution studies).

G CONSULTANTS

Dr. Marty O'Donnell, Ph.D., Professor of Chemistry: Allows the project to have access to his expertise in solid-phase synthesis of unnatural amino acids which are key intermediates in our overall synthesis.

Dr. Don Durden, M.D., Ph.D., Associate Professor of Pediatrics and Biochemistry: Allows the project to access his considerable expertise in vascular biology including $\alpha_v B_3$ integrin signal transductions and angiogenesis.

H CONTRACTUAL ARRANGEMENTS-CCTI is collaborating with Dr. Mark Green of Purdue University to accomplish this research proposal. This is arranged contractually as per the budget pages.

I LITERATURE CITED

1. Fan, T.P., Jaggar R., and Bicknell, R. Trends Pharmacol Sci 1995, 16 (2), 57.
2. Folkman, J. Nature Med. 1995, 27.
3. Brown, J.M., and Giaccia, J.A. Cancer Research 1998. 58, 1408.
4. Croix, B. St., Rago, C., Velculescu, V., Traverso, G., Romans, K.E., Mongomery, E., Lal, L., Riggins, G.J., Lengauer, C., Vogelstein, B., Kinzler, K.W. Science 2000, 289, 1197.
5. Kennel, S.J. and Mirzadeh, S. Nuclear Medicine & Biology 1998, 25, 241.

6. Dickman, S. Science 1998, 280, 1196.
7. Barinaga M, Science 1999, 283, 1831.
8. Arap, W., Pasqualini, R., Ruoslahti, E. Science 1998, 279, 377.
9. Pasqualini R, Kolvunen, E. , Ruoslahti, E. Nature Biotechnology 1997, 15, 542.
10. Arap, W. Pasqualini, R. Ruoslahti E., Current Opinion in Oncology 1998, 10, 560.
11. Pasqualini, R. Quarterly Journal of Nuclear Medicine 1999, 43, 159.
12. Blok, D. Feitsma, R., Vermeij, P., Pauwels, E.J.K. Eur J. Nucl. Med. 1999, 26, 1511.
13. Volkert, W.A., Hoffman, T.J. Chemical Reviews 1999, 99,2269 and references therein.
14. Seebach, D. and Hoyer, D. Angew. Chem. Int. Ed. 1999, 38, 1223.
15. Hirschmann, R., Nicalaou, K.C., et al. J. Am. Chem. Soc. 1993, 115, 12550.
16. Hirschmann, R., Nicalaou, K.C., et al. J. Am. Chem. Soc. 1992, 114, 9217.
17. Nicolaou, K.C., Trujille, J.I., and Chibale, K. Tetrahedron 1997, 53, 8751.
18. Peczuh, M.W. and Hamilton, A.D. Chem. Rev. 2000, 100, 2479.
19. Haubner, R. et al., J. Nucl. Med. 1999, 40, 1061.
20. Line, B.R., et al. J. Nucl. Med. 1999, 40, 79P, abstract number 319.
21. Keenan, R.M. et al. J. Med. Chem. 1997, 40, 2289.
22. Carron, C.P. et al. Cancer Research 1998, 58, 1930.
23. Haubner, R. et al. J. Nucl. Med. 2000, 41, 42P, abstract number 162.
24. Bock, M. et al. J. Nucl. Med. 2000, 41, 41P, abstract number 160.
25. See references in reference 19.
26. Personal communication, Renata Pasqualini, November 14, 2000.
27. Wong, A. Hwang, S.M., et al. Mol. Pharmacol. 1996, 50, 529.
28. Volkert, W.A., Hoffman, T.J. Chemical Reviews 1999, 99, 2269.
29. Caravan, P. Ellison, J.J. et al. Chemcical Reviews 1999, 99, 2293.
30. Molecular modeling was kindly provided by Prof. Lipkowitz and Dr. G. Lombardo both of IUPUI Chemistry Department using the X-ray crystal structure of Y-DOTA complex and applying the universal force field program to arrive at an energy minimized structure (see J.Am. Chem. Soc. 1992, 114, 10024).
31. O'Donnell, M. J., et al. J. Am. Chem. Soc. 1996, 118,6070.
32. Scott, W.L., et al. Tetrahedron Lett. 1997, 38, 3695.
33. Griffith, D.L., et al. Tetrahedron Lett., 1997, 38, 8821.
34. O'Donnell, M.J. et al. Tetrahedron 1999, 55, 6347.
35. Chemical & Engineering News, 2000, October 9, page 52.
36. Mammen, M. et al. Angew. Chem., Int. Ed. 1998, 37, 2754.
37. Burgess, K., and Lim, D., J. Med. Chem. 1996, 39. 4520.
38. Rose, K. J. Am. Chem. Soc. 1999, 121, 7034.
39. Fan, E. et al. J. Am. Chem. Soc. 2000, 122, 2663.
40. Kitov, P. I. et al. Nature, 2000, 403, 669.
41. Smith, J. W., and Cheresh, D.A. J. Biol. Chem. 1988, 263, 18726.
42. Mitjans, F. et al. J. Cell Sci. 1995, 108, 2825.
43. Pytela, R. et al. Science 1986, 231, 1559.

PURDUE UNIVERSITY

SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

[redacted]

Division of Research Grants
National Institutes of Health
Suite 1040
6701 Rockledge Drive MSC 7710
Bethesda, Maryland 20892-7719

RE: National Institutes of Health application entitled, "Chelate-Based Scaffolds in Tumor Targeting"

To Whom It May Concern:

The appropriate programmatic and administrative personnel of each organization involved in the application are aware of the PHS consortium grant policy and are prepared to establish the necessary inter-institutional agreements consistent with that policy. We understand that the grantee institution has the specific responsibility for ensuring that all required assurances are obtained.

Sincerely,

Mark A. Green, Ph.D.
Professor of Medicinal Chemistry

Diane Troyer, Assistant Director
Sponsored Program Administration

DIVISION OF NUCLEAR PHARMACY • DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
1333 HEINE PHARMACY BUILDING • WEST LAFAYETTE, IN 47907-1333
(765) 494-1441 • FAX: (765) 494-1414

PURDUE UNIVERSITY

SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

[redacted]

Joseph R. Garlich, Ph.D.
President
ComChem Technologies, Inc.
9731 Triboli Drive
Indianapolis, Indiana 46236

RE: Chelate-Based Scaffolds in Tumor Targeting

Dear Joe:

I am writing to confirm that my group is most interested in collaborating in ComChem's efforts to develop novel targeted chelate-based radiopharmaceuticals via application of combinatorial chemical techniques. We will be delighted to assist in your efforts to develop and evaluate radiopharmaceuticals targeted to tumor vasculature, as outlined in the accompanying subcontract.

We look forward to progress on this most exciting initiative.

Best regards,

Mark A. Green, Ph.D.
Professor of Medicinal Chemistry

MAG/ksk

DIVISION OF NUCLEAR PHARMACY • DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
1333 HEINE PHARMACY BUILDING • WEST LAFAYETTE, IN 47907-1333
(765) 494-1441 • FAX: (765) 494-1414

INDIANA UNIVERSITY

[redacted]

Joseph R. Garlich, Ph.D.
ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

SCHOOL OF MEDICINE

Dear Dr. Garlich:

This letter is to express my willingness to serve as a consultant on your NIH STTR Phase I F.L.A.I.R. proposal entitled "Chelate Based Scaffolds (Chelabody) in Tumor Targeting".

I agree to participate for three days of consultation at a minimum rate of $1000 per day in each of the two budget years.

Sincerely,

Donald L. Durden, M.D., Ph.D.
Associate Professor of Pediatrics,
Biochemistry and Molecular Biology
Herman B Wells Center for Pediatric Research
Attending Physician, Division of Oncology
Riley Childrens Hospital
Indiana University School of Medicine
Indianapolis, IN 46204

HERMAN B WELLS CENTER
FOR PEDIATRIC RESEARCH

James Whitcomb Riley
Hospital for Children
Indiana University
Medical Center
Cancer Research Institute
1044 W. Walnut Street
Room 402
Indianapolis, Indiana
46202-5225

317-274-8900
Fax 317-274-8679

INDIANA UNIVERSITY
PURDUE UNIVERSITY
INDIANAPOLIS

SCHOOL OF SCIENCE

IUPUI

[redacted]

Joseph R. Garlich, Ph.D.
ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

Dear Dr. Garlich:

This letter is to express my willingness to serve as a consultant on your NIH STTR Phase I F.L.A.I.R. proposal entitled "Chelate Based Scaffolds (Chelabody) in Tumor Targeting".

I agree to participate for three days of consultation at a minimum rate of $1000 per day.

Sincerely,

Martin J. O'Donnell

Martin J. O'Donnell
Professor

DEPARTMENT OF CHEMISTRY

402 N. Blackford Street
Indianapolis, Indiana
46202-3274

317-274-6872
Fax: 317-274-4701

http://chem.iupui.edu

SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

[redacted]

Joseph R. Garlich, Ph.D.
President
ComChem Technologies, Inc.
9731 Triboli Drive
Indianapolis, IN 46236

RE: Chelate-Based Scaffolds in Tumor Targeting

Dear Joe:

I am writing to confirm that I can provide training in bioassay techniques using equipment associated with the Purdue Combinatorial Chemical Biology Center, as needed in connection with ComChem's efforts to develop novel targeted chelate-based radio-pharmaceuticals via application of combinatorial chemical techniques.

I look forward to collaboration in this exciting initiative.

Best regards,

V. Jo Davisson

V. Jo Davisson, Ph.D.
Professor and Associate Head
(765) 494-5238 office
(765) 494-1414 fax
vjd@pharmacy.purdue.edu

VJD/jac

DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
1333 ROBERT E. HEINE PHARMACY BUILDING • WEST LAFAYETTE, IN 47907-1333
(765) 494-1403 • FAX: (765) 494-1414

Checklist

TYPE OF APPLICATION *(Check appropriate box[s].)*

[X] NEW application. *(This application is being submitted to the Public Health Service for the first time.)*

[ ] REVISION of previously-submitted application number ____________
(This application replaces a prior unfunded version of a new application.)

[ ] CHANGE of Principal Investigator *(if applicable)*
Name of former Principal Investigator ____________

1. ASSURANCES/CERTIFICATIONS

The assurances/certifications set forth below are made and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (small business concern) on the FACE PAGE of the application. Descriptions of individual assurances/certifications are found in application instructions under "Checklist." If unable to certify compliance with any item, provide an explanation and place it after this page.

• Human Subjects; • Vertebrate Animals; • Debarment and Suspension; • Drug-Free Workplace; • Delinquent Federal Debt; • Research Misconduct; • Civil Rights (Form HHS 690); • Handicapped Individuals (Form HHS 690); • Age Discrimination (Form HHS 690).

2. PROGRAM INCOME *(See discussion in application instructions under "Checklist.")*

All applications must indicate *(Yes or No)* whether program income is anticipated during the period for which grant support is requested.

[X] No [ ] Yes *(If "Yes," use the format below to reflect the amount and source(s) of anticipated program income.)*

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COSTS *(See discussion in application instructions under "Checklist.")*

Insert the rate, if known. If the applicant organization does not have a currently negotiated rate with the Department of Health and Human Services (DHHS) or another Federal agency, it must estimate the amount of indirect costs allocable (applicable) to the proposed Phase I project. That amount should be inserted in the space provided below. The applicant organization should also be prepared to furnish financial documentation to support the estimated amount, if requested by the Public Health Service. An applicant organization may elect to waive indirect costs if it so desires.

[ ] DHHS agreement, dated: ____________ - ____________ % salary and wages or ____________ % Total Direct Costs.

[ ] No DHHS agreement, but rate established with ____________, dated: ____________

[ ] Rate negotiation pending with the National Institutes of Health.

[ ] Indirect costs allocable (applicable) to this Phase I project are estimated to be $ ____________

[X] No indirect costs requested.

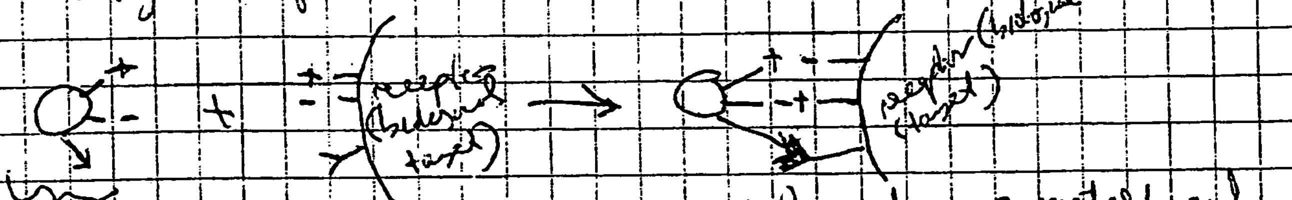
4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workplace and/or promote the non-use of tobacco products or have plans to do so?

[X] Yes [ ] No *(The response to this question has no impact on the review or funding of this application.)*

[redacted] IDEAS ON INFINITE AFFINITY BIOMOLECULAR TARGETING

We are developing chelating agents that bind to the $\alpha_v\beta_3$ integrin receptor. Such therapeutic/diagnostic drugs may be limited in their binding affinity to the receptor at low concentrations in the body. It would be advantageous if the small molecule therapy/diagnostic agent was designed to selectively covalently react with the receptor to insure the agent upon recognition of the receptor would bind covalently. This has been performed recently by Claude Meares (Proc. Natl. Acad. Sci. USA 98, p8480, 2001) in the context of ligands binding to antibodies where both are engineered to covalently react with each other upon binding. Because we cannot change the molecular target we cannot do this same thing. We can however use combinatorial chemistry to screen for reactive sites on the receptor that will react when our metal-ligand complex binds. This is illustrated below



DOTA based metal ligand complex in affinity for biological target (±) reactive group (→)

Receptor with complementary binding groups (±) & a potentially reactive (complementary) group (—<)

Interaction of metal ligand complex with complementary binding site of receptor/target brings the reactive group (→) into close proximity to target reactive group which ideally then will react together to form a new covalent bond.

This approach would allow long residence time of the drug (ie metal-ligand complex) at the target site without having to internalize the drug. This is particularly of value in treating diseases with short lived radioisotopes when you want to localize the radioactivity near the target tissue & have it stay there to deliver the whole dose to the target area

Continued on Page X

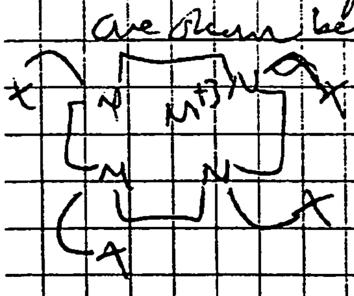
Read and Understood By

[illegible] Signed [redacted] Date

[illegible] Signed [redacted] Date

30
PROJECT A001 Notebook No. A001
Continued From Page X

[redacted] IDEAS ON TARGETING DRUGS TO TUMORS NECROTIC CENTER
Peregrine Pharmaceuticals has prepared antibodies that recognize internal antigens (see US Patents 4861581; 5019368; 5641750). These antibodies can't get into normal cells or even live cancer cells. They can get into necrotic tissue cells whose membranes are "leaky" or otherwise exposed to the circulating molecules. Antibodies are a poor choice to accomplish this as they are poorly diffusable into necrotic areas of cancers (solid tumors) because of their large size & often the inside necrotic areas of tumors are necrotic cause they are removed from the nutrients of the blood supply.
I propose an improvement over antibodies in this approach by using small molecules that recognize the proteins that are bound to DNA called histones or small molecules that recognize DNA itself.
Such small molecules would have radioisotopes (short lived) attached to them to do the therapy thus killing a solid tumor starting at the necrotic area.
Since histones are rich in lysines & arginines I propose small stable chelating agents that are polyanionic but that don't have affinity for bone mineral. Such small molecules are shown below schematically represented by DOTA-type chelators



where M^{+3} is a radiometal** with imaging and/or therapeutic properties & short half life and the "X" groups perform the chelation of the metal ion and also contain covalently attached polyanionic groups capable of recognizing histones*

Alternatively the histone recognizing groups (or DNA recognizing groups) can be on the tetraamine backbone or both the chelating arm and backbone.
Such constructs are envisioned for diagnosis & therapy of solid tumors that possess at least some necrotic cells which allow localization.

*or polycationic groups that recognize & bind strongly to DNA] **or M^{+3} is a reactive oxygen species catalyst generating & nonredundant such as Fe^{+2}/Fe^{+3}
Continued on Page X

Read and Understood By

Joseph R Sav — Signed | [redacted] Date | Signed | [redacted] Date

PROJECT

[redacted] IDEAS ON ENHANCING RADIOTHERAPY

I propose introducing an iron complex in conjunction with radioisotope therapy to increase the potency of the radiation via iron catalyzed free radical generation.

Such complexes could be attached to target localizing molecules such as peptides, antibodies, growth factors, or other proteins.

The iron complex would consist of Iron (either +2, or +3 valence state) chelated by an organic polydentate ligand. It is critical that the iron not be coordinatively saturated so it can generate reactive oxygen species in the presence of ionizing radiation.

Such ligands for complexing the iron can be amino carboxylates, amino phosphonates, phosphonates, and mixes thereof & includes half esters of phosphonic acids.

Such complexes & their targeting groups are preferenced to enter ~~to~~ target cells such as tumor cells to maximize effectiveness.

Such an approach would be an addon to ~~chemotherapy.~~ radiotherapy using ionizing radiation.

I also propose ablating the bone marrow using such iron complexes even without ionizing radiation for treatment of multiple myeloma & other diseases & for example destroying the marrow prior to a bone marrow transplant including the use of gene therapy (i.e. altered transplanted stem cells).

Continued on Page X

Read and Understood By

Joseph R Garlich — Signed | [redacted] — Date | Barry [signature] — Signed | [redacted] — Date

JECT mirrodoge p84,85,86,87 Notebook No. A001

Continued From Page —

[redacted] Documentation of Disclosure to USPTO #468887 dated by them as Feb 8, 2000 (cover page this page & 3 pages submitted to USPTO as p85,86,87 that follow; (this page reduced by copy machine to 80% of original & next 3 pages reduced to ~~80~~ 85% of their original size;

OIPE JC82 [redacted] PATENT & TRADEMARK OFFICE

DISCLOSURE DOCUMENT NO.
468887
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

February 3, 2000

Document Disclosure Program
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

The undersigned, being the inventor of the disclosed invention, requests that the enclosed papers, 3 pages be accepted under the Disclosure document Program, and that they be preserved for a period of two years.

Enclosed is a Check for $10.00 to cover this submission. Thank you for your help.

Sincerely,

Joseph R Garlich

Joseph R. Garlich, Ph.D.
328 West Columbine Lane
Westfield, IN 46074

Continued on Page 85

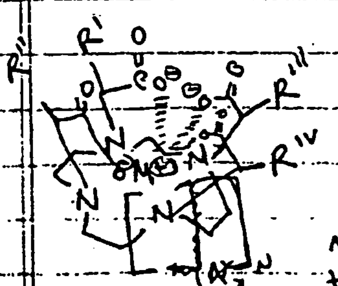
Read and Understood By

Joseph R Garlich | [redacted] | Barry Drueke | [redacted]
Signed | Date | Signed | Date

MACROCYCLIC MIMOTOPES USEFUL FOR Page 1
DRUG TARGETING (Therapy) OR IMAGING IN MEDICINE of 3
by: JOE GARLICH. Joseph R Garlich Feb 3, 2000

Mimetopes are peptides that mimic the structure of a folded protein. The spatial orientation of molecular binding interactions are ~~cruc~~ crucial to molecular recognition events such as antibody recognition of a substrate, protein recognition of substrate. It is important for the proper spatial orientation to be a low energy form or otherwise preferred orientation. I propose using macrocyclic chelating agents which when complexed with appropriate imaging or therapeutic metal ions will form a conformationally restricted spatially oriented presentation of molecular recognition units (such as peptides, H-bond donors/acceptors, charge-charge interaction, lipophilic-lipophilic, hydrophilic-hydrophilic, pi bond stacking, Van der Waals interaction etc) that are useful in medicine for example binding selectively to membranes of cancer cells or other target cells or proteins. Some specifics are below:

1a) Macrocycles such as 1,4,7,10-tetraazacyclododecane are good for this purpose as the chelating "arms" (ie Nitrogen pointing substituents) are all situated on one "side" of molecule & held (locked) into conformation thru bonding with metal ion: (Non-limiting example)



where $R^I, R^{II}, R^{III}, R^{IV}$ are independently chosen molecular recognition units or elements as illustrated above that together when complexed with a metal ion form spatial collection that recognizes & binds preferentially to some target protein or cell membrane

N = 0, 1 preferably to give cyclononane-triaza- cyclododecane-tetraaza

Continued on Page 86

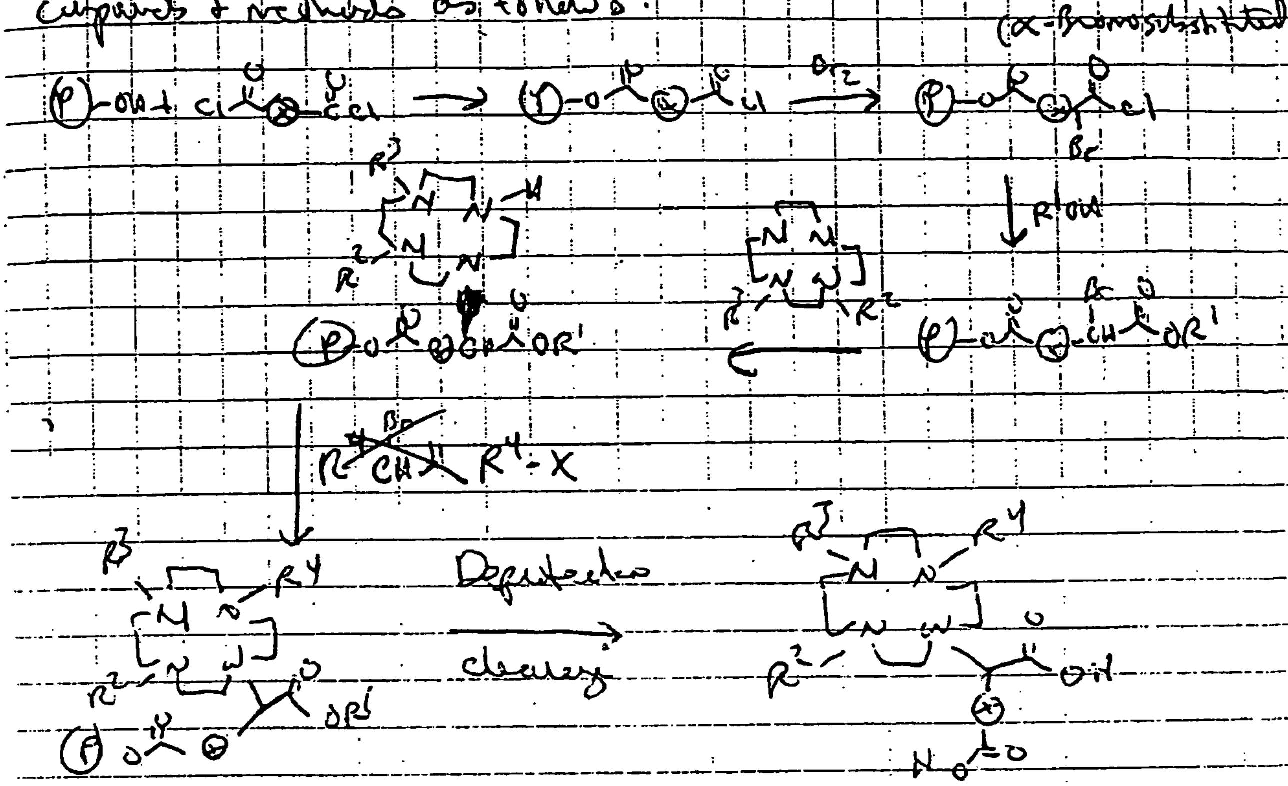
Read and Understood By

Joseph R Garlich [redacted]
Signed Date

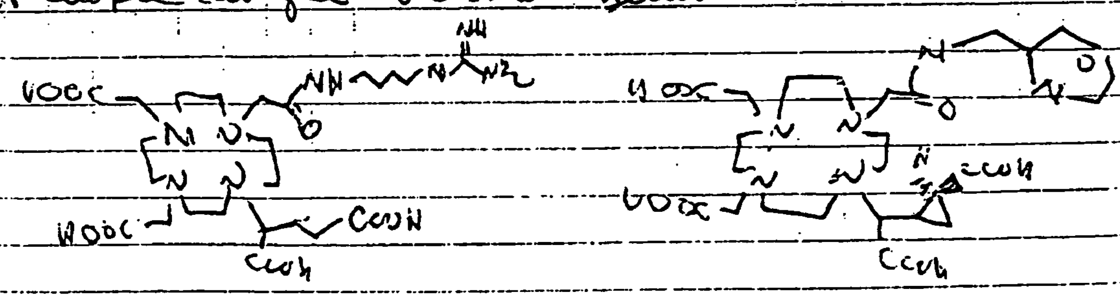
Barry [illegible] [redacted]
Signed Date

IDEAS ON MORE CHELA BODIES:

For mimicking Antibody recognition of the $\alpha_v\beta_3$ receptor I propose compounds & methods as follows:



Where R4 has an basic amino group in it.
When (P) = polymer resin for solid phase synthesis
Where (X) is a spacer group allowing for optimal geometric positioning of the acid & basic groups in space.
Where R3 & R2 are acetate chelating arms.
A Couple examples are shown below:

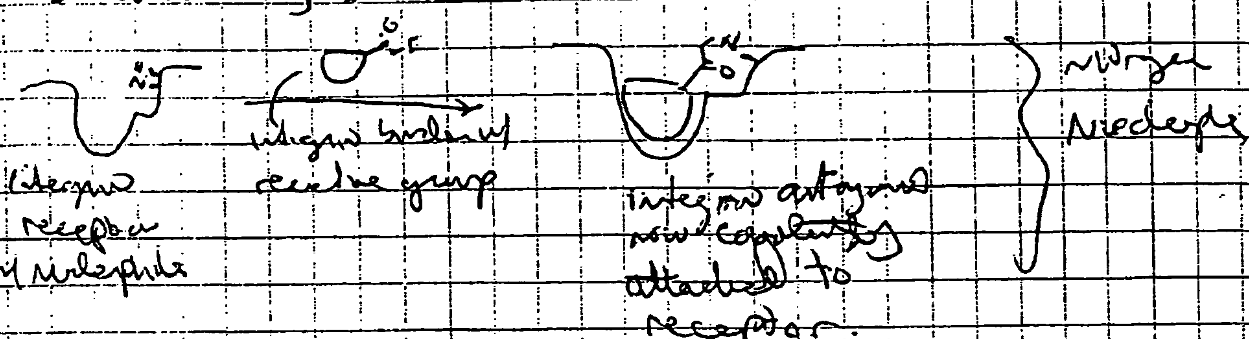


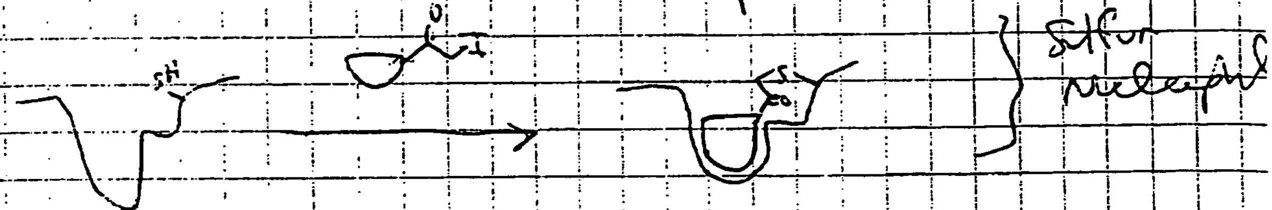
The ligands and metal complexes thereof are novel compositions. Such complexes are envisioned to be antagonists of $\alpha_v\beta_3$ & other integrins & be useful imaging & therapeutic agents

Signed [redacted] Date
Signed [redacted] Date

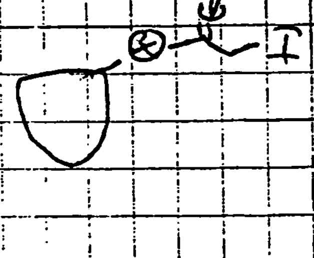
IDEAS ON INFINITE AFFINITY INTEGRIN BINDERS:

I propose DOTA based chelating agents that also contain a reactive chemical group that is not reactive until the chelating agent (with metal complexed) binds to the target for example an integrin receptor such as $\alpha V\beta_3$. The ultimate binding lowers the activation energy & the reactive group can then react. This is shown schematically below:





This invention takes advantage of the nearby amino acid groups to the binding pocket. One can probe & find such nearby groups by introducing a variable spacer linker between the reactive group and the receptor binder as shown diagrammatically below



X can be aliphatic/aromatic carbon chain to space & orient the reactive group in a certain distance & direction.

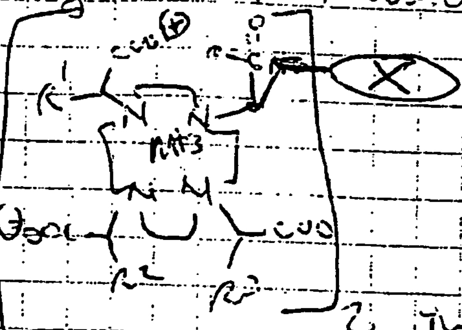
Continued on Page

Read and Understood By

Signed: Joseph R Barr Date: [redacted] Signed: [signature] Date: [redacted]

IDEAS ON DIMERIC CHELABODIES:

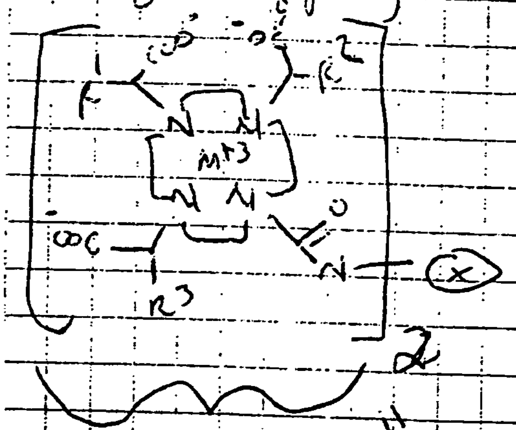
I propose the following dimeric types of metal complexes as targeting agents for the delivery of diagnostic & therapeutic agents to the neovasculature of tumors/cancers:



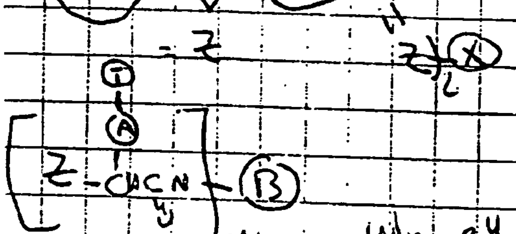
where R^1, R^2, R^3 are organic moieties that bind with certain receptors such as $\alpha v\beta 3$ integrin receptor & R^1, R^2, R^3 are independently chosen. M^{+3} is the diagnostic or therapeutic metal ion such as but not limited to Y-90, Ho-166, Gd

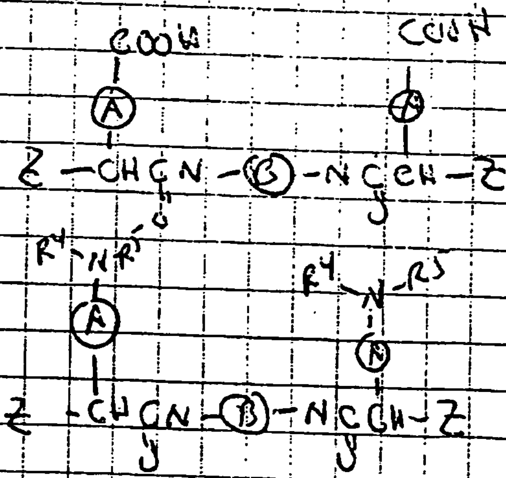
2 The (X) group serves as a spacer group to hold the molecular recognition elements bound to the complex in the proper spatial separation for good binding and/or good internalization upon binding.

Alternatively one of the acetate arms can be used as an amide linkage to simplify the synthesis to give generically:



Specific examples are:





where R^4 or R^5 are groups to improve the binding of the above

where (T) is either an acid group or a basic group and the N denotes the number of groups in brackets attached to core spacer group (B)

where (A), (B) are organic spacer groups (alkyl, arylalkyl, heteroaromatic, oxyethylene, etc.) to optimize binding &/or internalization after receptor binding

Continued on Page X

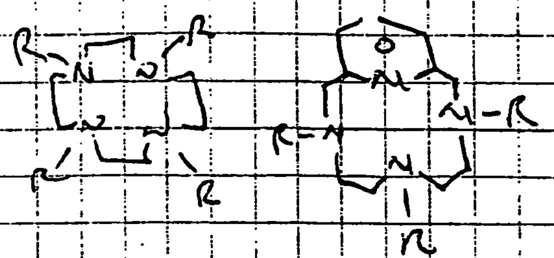
Read and Understood By

Signed [redacted] Date

Signed [redacted] Date

[redacted] IDEAS on Chelabodies:

I have previously described Chelabodies as a way to mimic antibody molecular recognition. I now propose a few new structures that also fall under this concept:



where R groups are selected independently from the following groups:

→ CH_2COOH

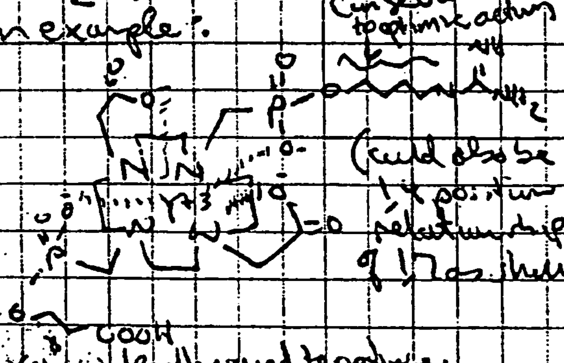
$-CH(R^1)P(O)(OH)OR^2$ when R^1 & R^2 contain organic binding groups or R^1 contains an organic binding group & R^2 is a simple methyl, ethyl, propyl etc.

$-CH_2P(O)(OH)OR^1$ when R^1 contains an organic binding group (such as COOH or guanidine for RGD mimetics)

$-CH_2P(O)(OH)R^1$ when R^1 contains an organic binding group (such as COOH or guanidine for RGD mimetics)

$-CH_2C(O)NR^1-OH$ R' as above

An example:



(could also be 1,4 position relationship instead of 1,7 as shown)

(RGD mimic)

Continued on Page X

Read and Understood By

[redacted] Signed [redacted] Date

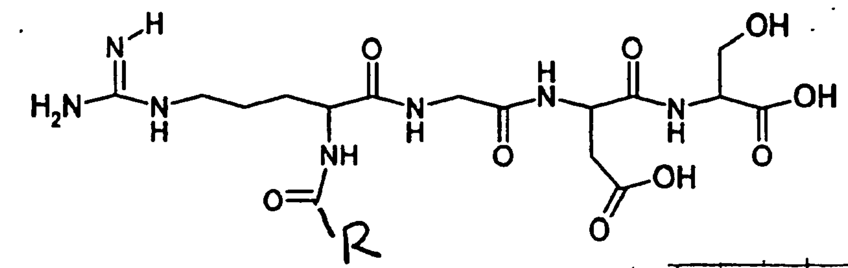
Barry Ancker Signed [redacted] Date

[redacted]

PROJECT ______ **Notebook No.** A027

Continued From Page —

[redacted] Preparation of Phosphate buffered solutions for Products to evaluate to quantitate their ability to be $\alpha_v\beta_3$ receptor antagonists with the designed ability to covalently bind to the receptor after initial noncovalent binding to the protein that recognizes the RGD motif.



(See structures next page)

New Solution Lot #	Maria Prep Lot #	Bob's Synthesis Lot #	Mol Wt	Weighed amount (mg)	uMoles	Vol to = 1mM of PBS* (mL)	Retention Time** (mins) LC	MH Run
A027-3A	A023-88 A	A025-74	544.53	1.3	2.39 uMole	2.388	1.964	54[illegible]
A027-3B	A023-86 B	A025-76	658.41	0.5	0.76	0.760	2.544	659
A027-3C	A023-84 B	A025-78	611.41	0.5	0.82	0.820	2.328	611/61[illegible]
A027-3D	A023-90	A025-72	532.56	1.1	2.07	2.065	0.708	533

All of these structures represent reduction to practice of an $\alpha_v\beta_3$ binding motif (i.e. rgds) covalently bound with an electrophilic group capable of covalent attachment to the cysteine group (or other nucleophilic group) on the $\alpha_v\beta_3$ receptor in vivo & serve as targeted therapeutic that interferes in angiogenesis.

* PBS = 5mM used on p. A024-97

*

Continued on Page 4

Read and Understood By

Signed [illegible] Date [redacted] Signed [illegible] Date [redacted]

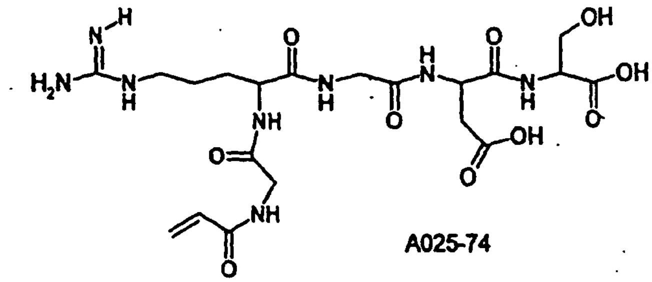
PROJECT

Notebook No. A027

Continued From Page 3

CONFIDENTIAL COMCHEM TECHNOLOGIES APRIL 28 2003 CONFIDENTIAL

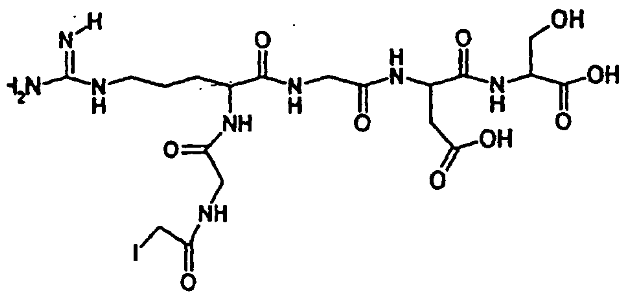
2.390 mL of Solution of 1mMolar Ligand (lot A023-88A) in PBS; lot= A027-3A



Molecular Weight =544.53
Exact Mass =544
Molecular Formula =C20H32N8O10

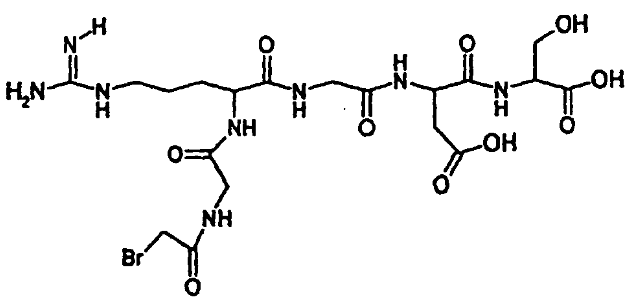
A025-74

0.760 mL of Solution of 1mMolar Ligand (lot A023-86B) in PBS; lot= A027-3B



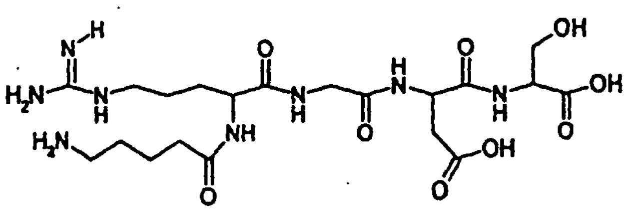
Molecular Weight =658.41
Exact Mass =658
Molecular Formula =C19H31IN8O10

0.820 mL of Solution of 1mMolar Ligand (lot A023-84B) in PBS; lot= A027-3C



Molecular Weight =611.41
Exact Mass =611
Molecular Formula =C19H31BrN8O10

2.065 mL of Solution of 1mMolar Ligand (lot A023-90) in PBS; lot= A027-3D



Molecular Weight =532.56
Exact Mass =532
Molecular Formula =C20H36N8O9

Continued on Page X

Read and Understood By

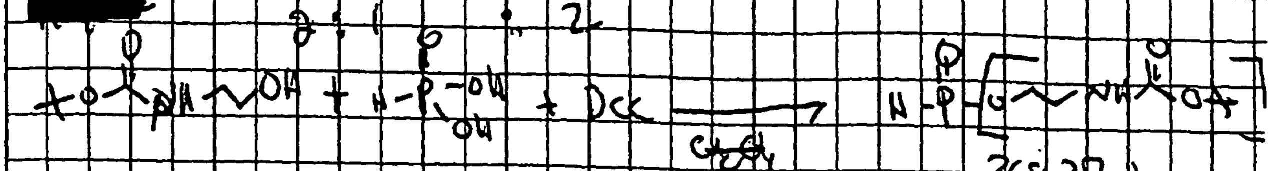
Signed [redacted] Date

[redacted]

PROJECT ____________ Notebook No. N01

Continued From Page —

[redacted]



161.20	3.07 mMole	6.15 mMole THF	368.37 g/mole
d=1.042	Aldrich	Aldrich	$C_{14}H_{29}N_2O_7P$
981 mg	82.00 g/mole	206.33 g/mole	
6.15 mMole	252 mg	1.268 g	

Wrme dissolve w/ 10 ml THF; add H_3PO_3 & stir 5 min. Weigh out DCC & Dissolve in 10 ml THF & add all at once to solution. start 3pm 9/7/02 heavy ppt occurs within 15 seconds. continue stirring at room temp. Mon 9/9/02 at 12 noon (45 hrs) let settle & take one of supernatant + 1 ml MeOH & run 31P NMR - 2971. Shows mostly (+90%) of the desired product (Bis-phosphite) & ~10% of monophosphite. Vacuum filter wash w/ CH_2Cl_2 & rotovap. Dry under house vac until 7pm. Clear oil wt = 72.9354 - 71.8613 = 1.0741 g = 95
Theoretical wt = ~~6.15~~ 3.07 mMole x 368.37 = 1.13 the

Only trace of mono ester formed (<9%) So is 91% Bis ester

Continued on Page

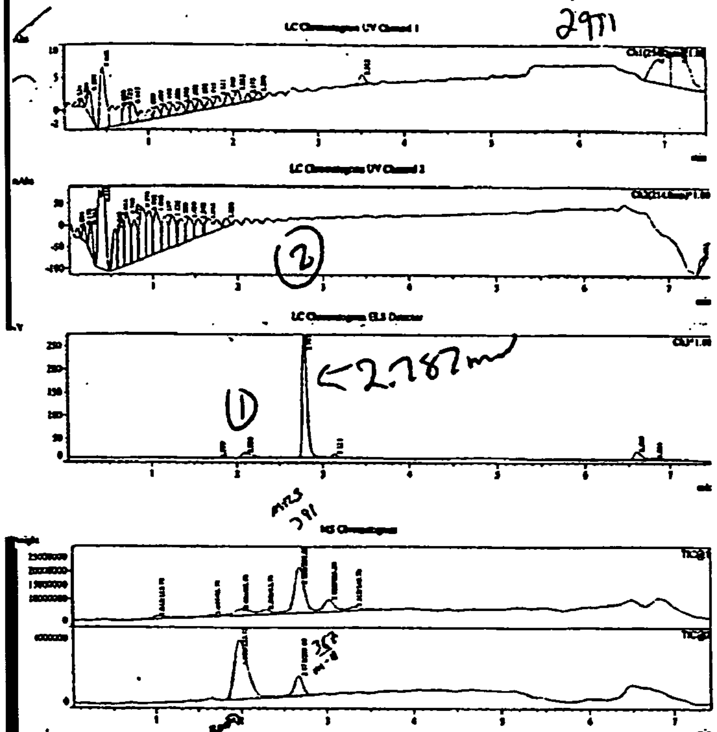
Read and Understood By

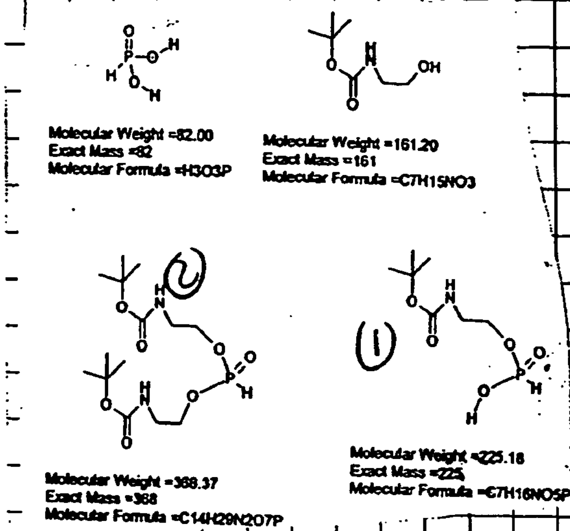
Signed [redacted] Date

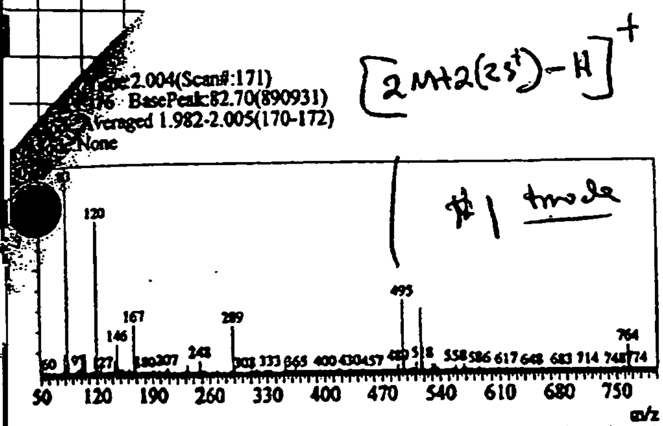
Signed [redacted] Date

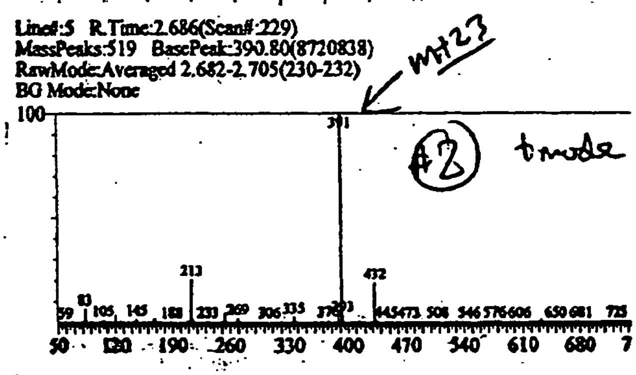
JECT ______________________________ Notebook No. A023

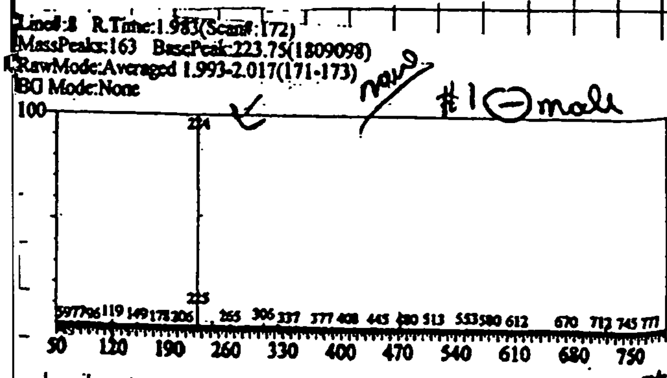
Continued From Page 29









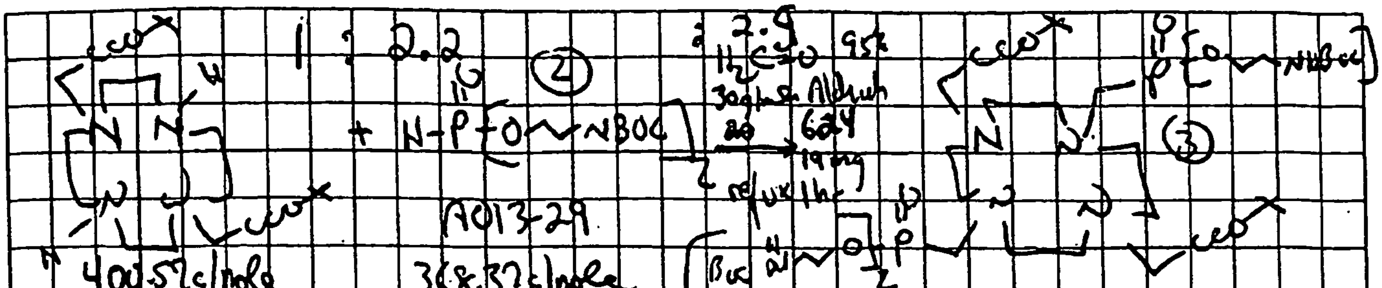


	Ret time	UV	MW
#2	2.787	—	391 (m+ / 367 (M-H
#2	2.096	—	M-H=224

Continued on Page

Read and Understood By

[signature] [redacted] [signature] [redacted]



400.52 g/mole
100mg = 250 uMoles
(1)

368.32 g/mole
549 umoles
202mg 549uM

1161.33 g/mole
$C_{50}H_{98}N_8O_{18}P_2$

17% isolated yield
(23% isolated yield of mono sub)

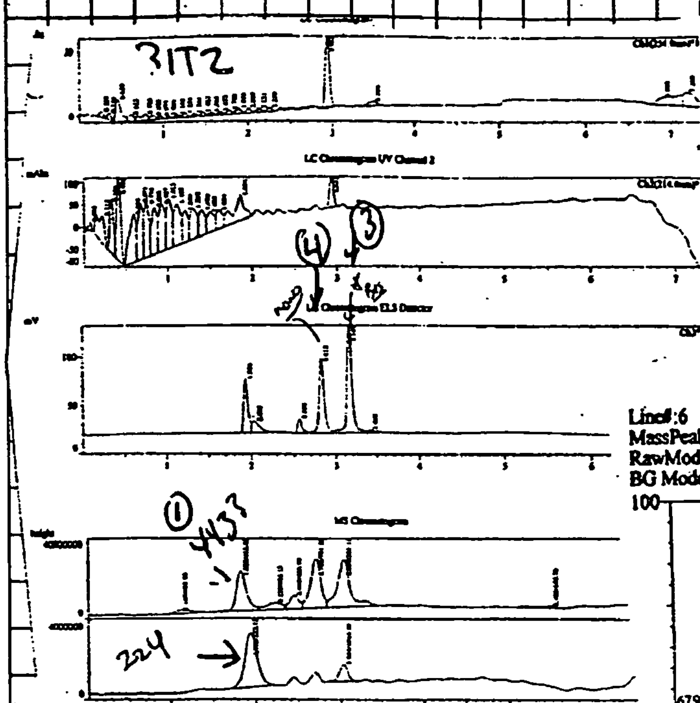
Adapted from Synthesis, 1985, p453
(tare 25ml flask = 19.9882g)
Mix bis cyclen + phosphite in benzene (8 mL) and added dry paraformaldehyde all at once. Remove 10uL slurry & add 400uL MeOH crude LCMS = 3T10. Heat w/ stirring in 25ml RB flask w/ reflux condenser at 8pm mostly at 8:30 all soluble (paraformaldehyde in soln) & is pale yellow; remove 10uL blow down w/ argon, add 400uL MeOH = 3T11 LCMS indicates substantial amounts of mono (ret 2.x min MH = 781) and bis-reacted product (ret time ELS 3.148 min MH = 1161 & M+23 1183)! First evidence for a chelate body that is capable of bridging between two phosphonic acid groups. Put back on heat (reflux bath temp = 91°C benzene bp = 80°C) 8:45pm – 9:15pm (another ½ hour). Remove last blow down w/ argon & dissolve in 400uL MeOH LCMS = 3T12; looks like a little more product formed but very little of phosphite left. Put in fume hood & let evaporate. color: still liquid left. blow down w/ argon to thick oil; put on high vacuum & foams up. wt = 18.2517 − 17.9923 = 259.4g (290 theory = 89% of theory wt).
Mary prepped & isolated 48.5mg of the bis substituted (3) ~17% = A016-15
isolated 45mg of the mono (4) = A016-15A = 23%
~40% yield split between 2 products

Continued on Page 32

PROJECT

Notebook No. A013

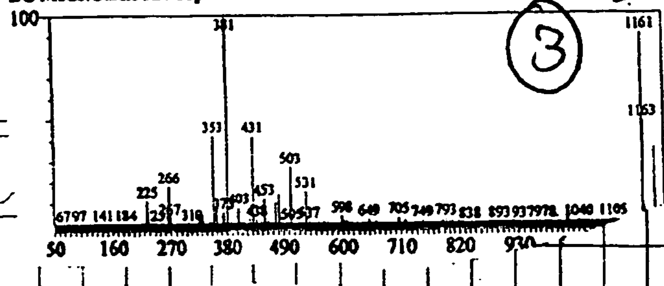
Continued From Page 31



Line#:6 R.Time:3.039(Scan#:261)
MassPeaks:581 BasePeak:381.15(2984132)
RawMode:Averaged 3.020-3.067(259-263)
BG Mode:Calc.Peak Top

MW-1161

(3)

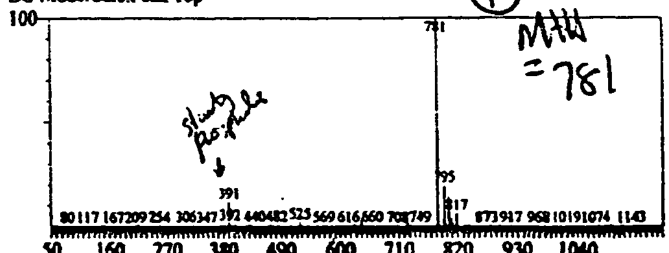


ret time ees 3.142

Line#:5 R.Time:2.705(Scan#:231)
MassPeaks:561 BasePeak:781.25(6550077)
RawMode:Averaged 2.670-2.717(229-233)
BG Mode:Calc.Peak Top

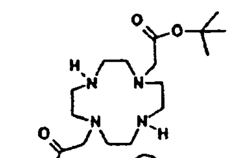
(4)

MW = 781



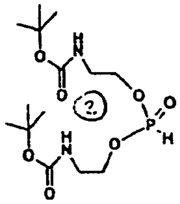
ret time ees 2.8

A013-31



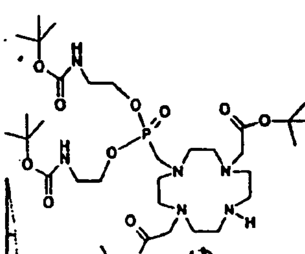
(1)

Molecular Weight =400.57
Exact Mass =400
Molecular Formula =C20H40N4O4



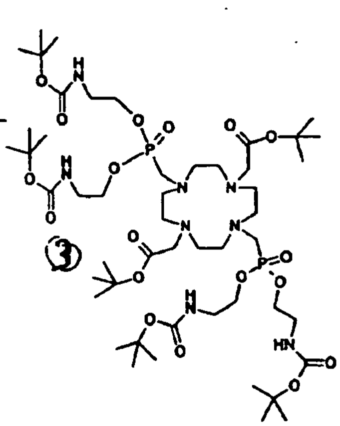
(2)

Molecular Weight =368.37
Exact Mass =368
Molecular Formula =C14H29N2O7P



(4)

Molecular Weight =780.95
Exact Mass =780
Molecular Formula =C35H69N6O11P



(3)

Molecular Weight =1161.33
Exact Mass =1160
Molecular Formula =C50H98N8O18P2

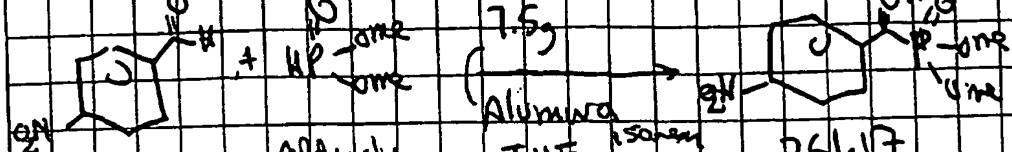
Continued on Page X

Read and Understood By

Joseph Nau — Signed | [redacted] — Date

Barry ... — Signed | [redacted] — Date

[redacted] 1.5 : 1



7.5 g Alumina, THF, 1 solvent

Aldrich 151.12 g/mole mp 105 — Aldrich Dimethyl phosphite bp 170°, 110.05 g/mol, d = 1.20 g/mL — $C_9H_{12}NO_6P$ 261.17

2g = 13.2 mmole — 8.8 mmol 969 mg = 808 uL

Boeden Synthesis 1982, p 916: use Brockmann Grade I basic alumina (0.5 g per millimole. Dissolve in 60 mL of THF & add alumina. Then added phosphite. Start 10 am Thurs 9/19/02, RT in orbital shaker at 100 rpm. Sat 9/21 orange color has turned to bright yellow (T = 37 hrs) let set & remove 5 mg of supernatant + 400 uL MeOH = 53T1

See desired product at retention of ~1 min. Filter thru sponge into crystallizing dish & let evap (tare 85.6602)

Add 5 mL CH_2Cl_2 some soluble & not all; decant & repeat 2x.

(A) = CH_2Cl_2 soluble = 70.9459 − 69.6900 = 1.2559 g sample some into MeOH + 53 — yellow solid

(B) = INSOLUBLE wt = 86.7870 − 85.6602 = 1.1268 g yellow solid sample some into MeOH = 53B

B is pure material & A is impure.

— nylon ship —

Rt time (e)(s) = 1.983 min 98% pure (UV)

Gave to Bob to make tosylate (see A015-28)

Continued on Page

Read and Understood By

Joseph R Barber — [redacted] — Benjamin [illegible] — [redacted]

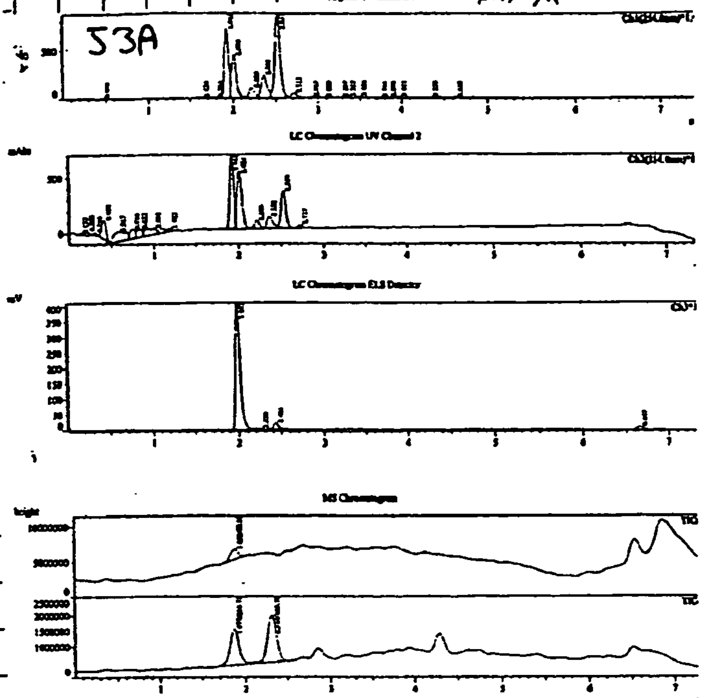
Signed — Date — Signed — Date

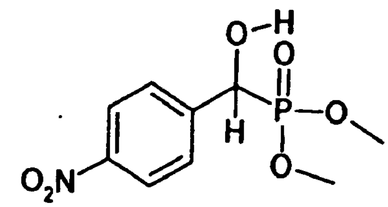
PROJECT

Notebook No. A013

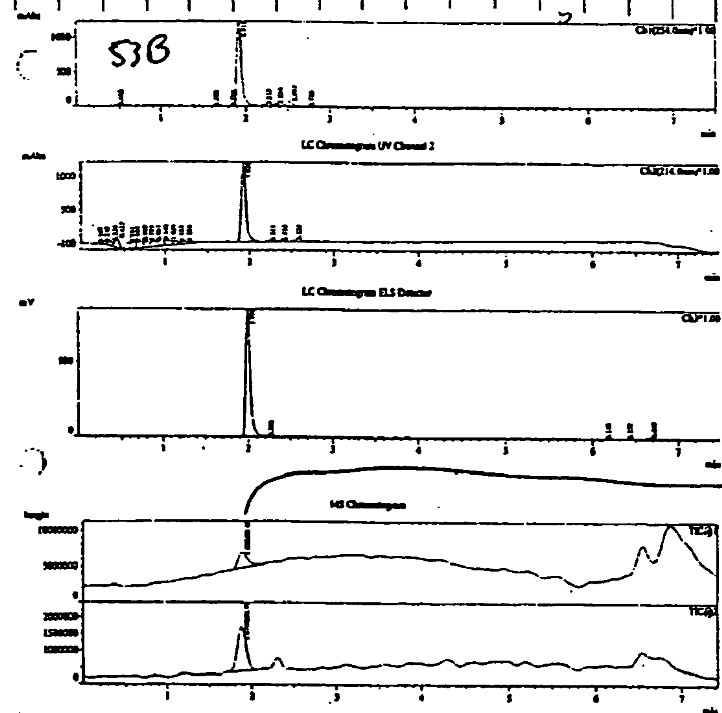
Continued From Page 53

Water gradient method A013-1A





Molecular Weight =261.17
Exact Mass =261
Molecular Formula =C9H12NO6P



53B pure

303

M+41 = 302 +H = 303

m-H = 259(?) are lower than expected

Continued on Page

Read and Understood By

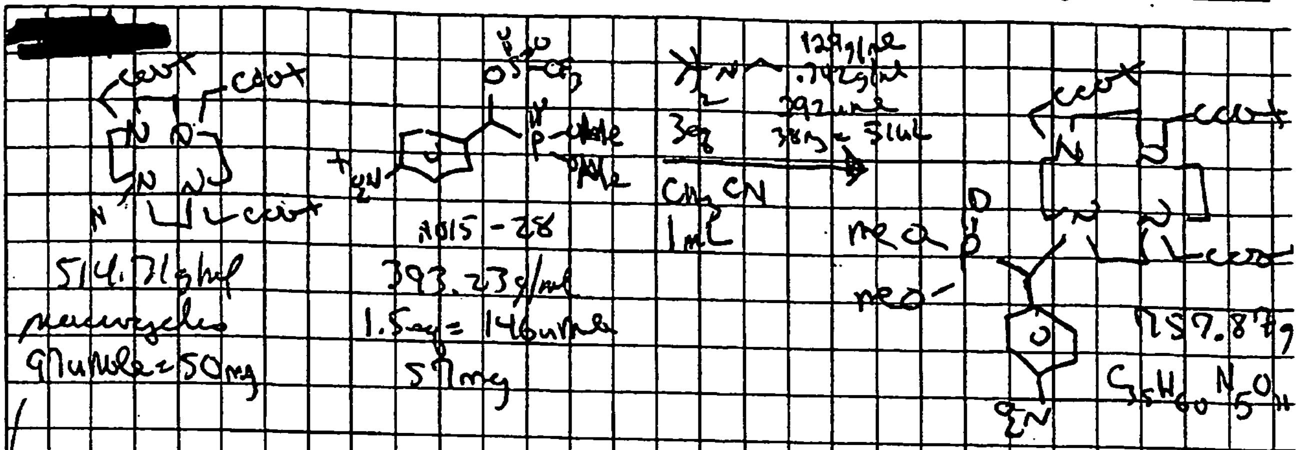
Signed [redacted] Date

Signed [redacted] Date

PROJECT ______ Notebook No. A013

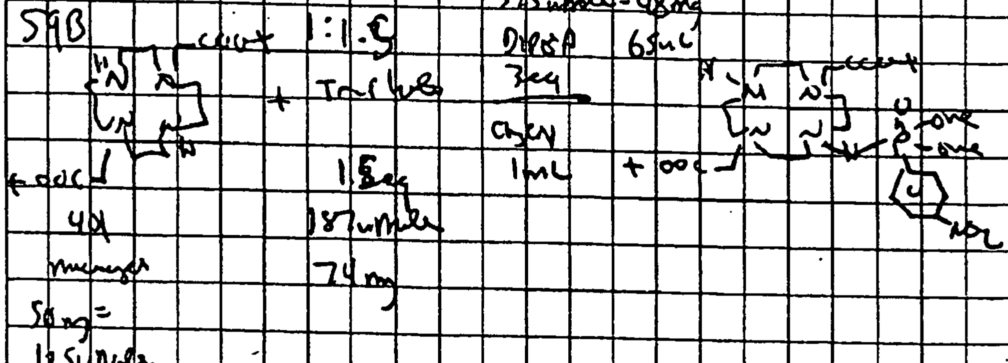
Continued From Page —

[redacted]



Dissolve in 1 mL dry CH_3CN. Add 51 uL DIPEA, add 57 mg solid triflate. Stirring 10 pm 9/25/02 Wed – all soluble & turns blood red when triflate added! Very be anion forming. @ 9/26 7 am take 10 uL + 40 uL water = 59T1 1083 still present! Tosylate almost all gone – trace still at 3.5 min & new UV peaks at 2.2 & 2.3 min; 1st peak not shows 370, 433, 574 m/z in positive mode & in neg mode second peak shows strong 66, 355.

Tosylate appears to have done something else besides desired reaction. Trace mass M+D = 758 observed at 2.9 min (ELS); shows 3 – loss of t-butyl (-56) peaks 702, 646 and 587. Also see M+23 = 780. Essentially no reaction!



Combine all in 1 dram vial. Start 10 pm Thurs 9/26; Fri 9/27 8 am (10 hrs) remove 10 uL + 40 uL MeOH = 59BT1; ~~add~~ slight reaction; add 74 mg more Tosylate 2 pm Fri 9 – no obvious color change (still dark red but gets noticeably darker). Sat 2 9/28 sample like + MeOH 59BT2; more product present & unknown peak at ~2.3 smaller; Tosylate still present & starting DIEA added – keep stirring Monday 9/30 8 am 10 uL + 40 uL MeOH = 59B3;

Continued on Page 6

Read and Understood By

Signed [redacted] Date

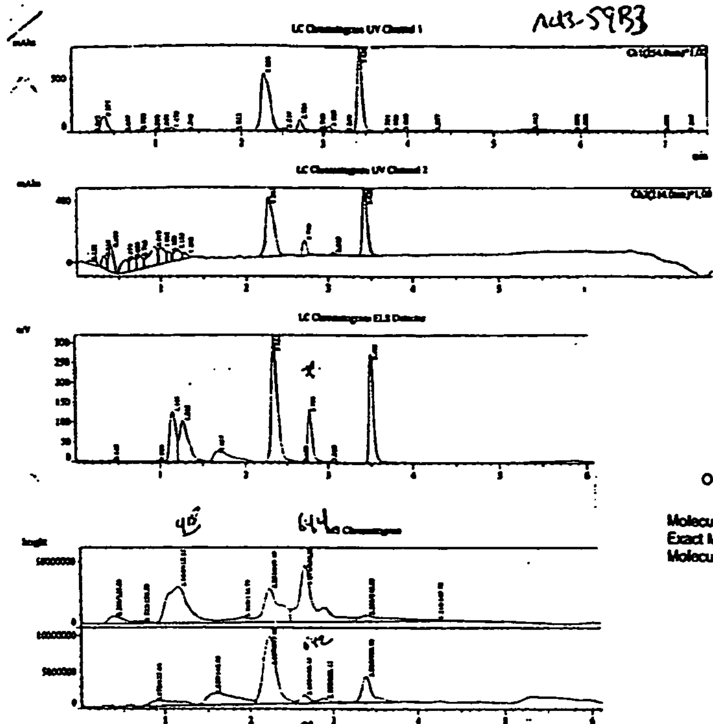
Signed [redacted] Date

Notebook No. A017

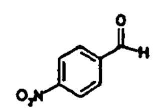
Continued From Page 59

Waters Method A013-1A (Standard method)

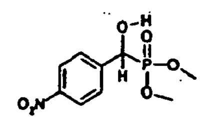
A013-59B3



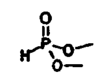
A013-59B



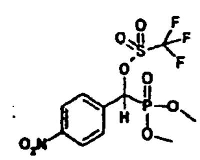
Molecular Weight =151.12
Exact Mass =151
Molecular Formula =C7H5NO3



Molecular Weight =261.17
Exact Mass =261
Molecular Formula =C9H12NO6P

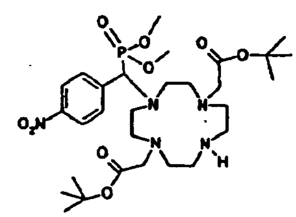


Molecular Weight =110.05
Exact Mass =110
Molecular Formula =C2H7O3P

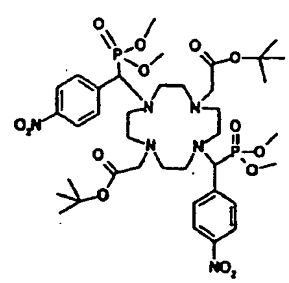


Molecular Weight =393.23
Exact Mass =393
Molecular Formula =C10H11F3NO8PS

MH+ = 644
M-H 642

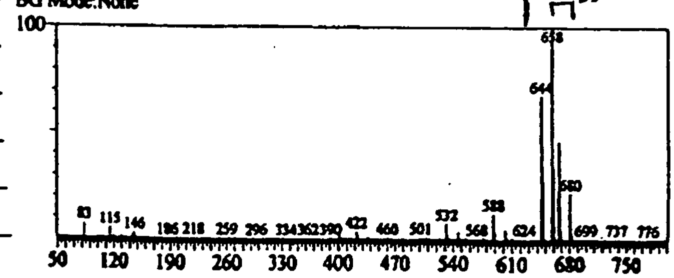


Molecular Weight =643.72
Exact Mass =643
Molecular Formula =C29H50N5O9P



Molecular Weight =888.88
Exact Mass =888
Molecular Formula =C38H60N6O14P2

Line#:6 R.Time:2.671(Scan#:229)
MassPeaks:573 BasePeak:658.20(9039883)
RawMode:Averaged 2.658-2.682(228-230)
BG Mode:None



Continued on Page X

Read and Understood By

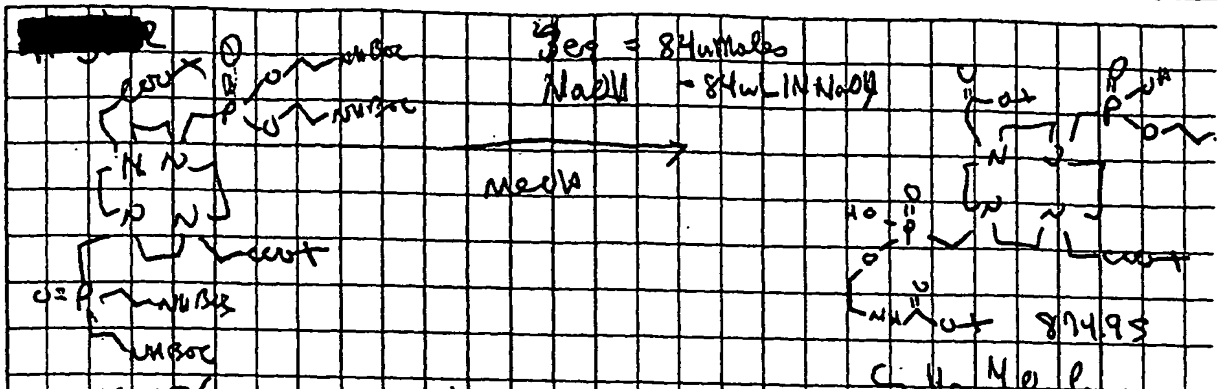
Signed | Date [redacted] | Signed | Date [redacted]

PROJECT

Notebook No. A013

Continued From Page —

[redacted]



A0016-15 (prep of A013-31)
1161.33
24.25mg = 20.9umoles
→ Dissolved all of A0016-15 in 1.0 mL MeOH; dissolve & (while stirring) add 4 eq of NaOH (84uL of 1N NaOH w/ H_2O). - gave turbid; add 200uL
start 10pm Wed 9/25/02 — mono - still cloudy - turbid - stir overnite.
Thur 9/26 7am - cloudy - take 10uL say MIX 1200uL H_2O/200uL MeOH = no visible. significant hydrolysis has taken place! (traces of 1018 & mostly prd is at longer retention time!! at 8pm check again = 6372; looks like some hydrolysis going on. keep stirring at room temp; Fri 8am 6373 still no sign of desired bis-cleaved ester — sign of a t-butyl ester cleaved!! Monday 9/30/02 6374; still a mess! Heat in hot bath at 62°C start (add 100 uL 1N NaOH = 5eq) overnite - all soluble at 8am 10/1 next morning. dilute concentrate H_2O see peak for 875 at 2.664 = 63
10-4-02 white precipitate / let settle dilute and supernatant + 400 H_2O = 63HT:
still some 875 but larger amount of 819 = loss of t-Butyl. So can selectively cleave phosphonate ester without cleaving the t-butyl ester or acetic ester acid!. add 100uL 1N NaOH heat at 90-93°C. Start 10am ~~10-5-02~~ 4pm 10-6-02; Stop 4pm 10-7-02. leave at room temp until 10-15-02; dilute 10uL + 800uL = 6347 in MeOH (some ppt present in vial cause no NHBoc)
↳ looks like some mono ester still left & some of both ester groups remain

Continued n

Read and Understood By

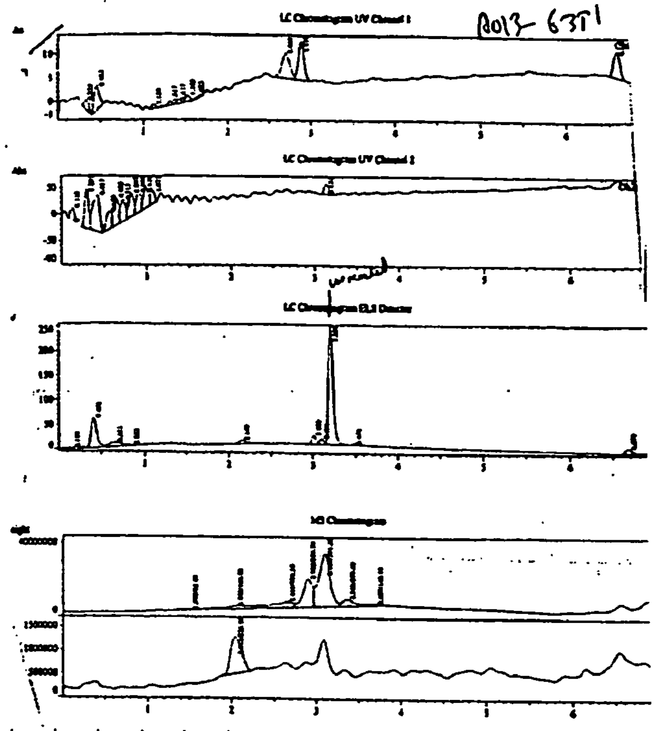
Signed [redacted] Date Signed [redacted]

PROJECT

Notebook No. A013

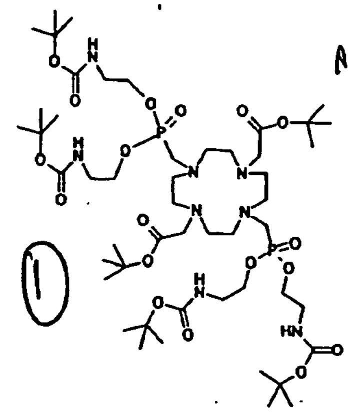
Continued From Page 63

A013-63T1



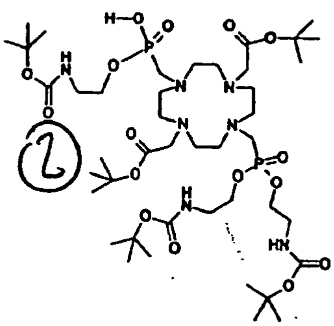
A013-63

1



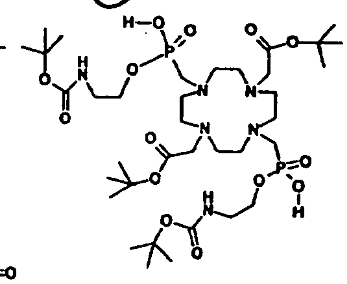
Molecular Weight =1161.33
Exact Mass =1160
Molecular Formula =C50H98N8O18P2

2



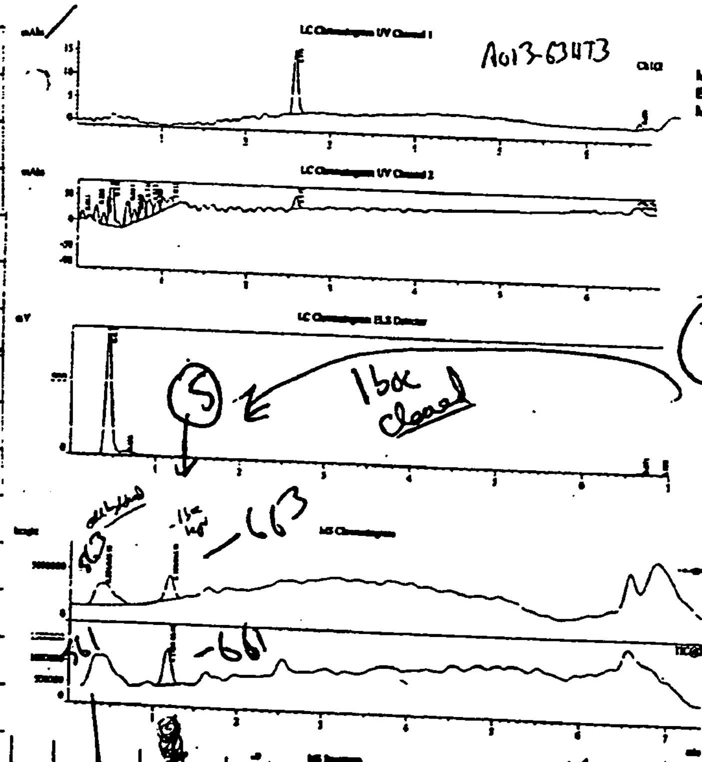
Molecular Weight =1018.14
Exact Mass =1017
Molecular Formula =C43H85N7O16P2

B



Molecular Weight =874.95
Exact Mass =874
Molecular Formula =C36H72N6O14P2

A013-63HT3



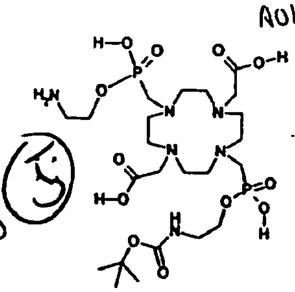
5 ← 1 boc cleaved

-663

-661

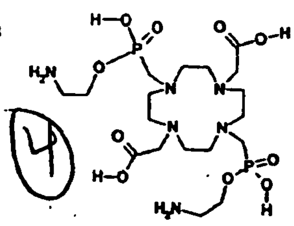
A013-65

5



Molecular Weight =662.62
Exact Mass =662
Molecular Formula =C23H48N6O12P2

4



Molecular Weight =562.50
Exact Mass =562
Molecular Formula =C18H40N6O10P2

Base cleavable Boc Group!

4!

C ntinued n Page 8

Read and Understood By

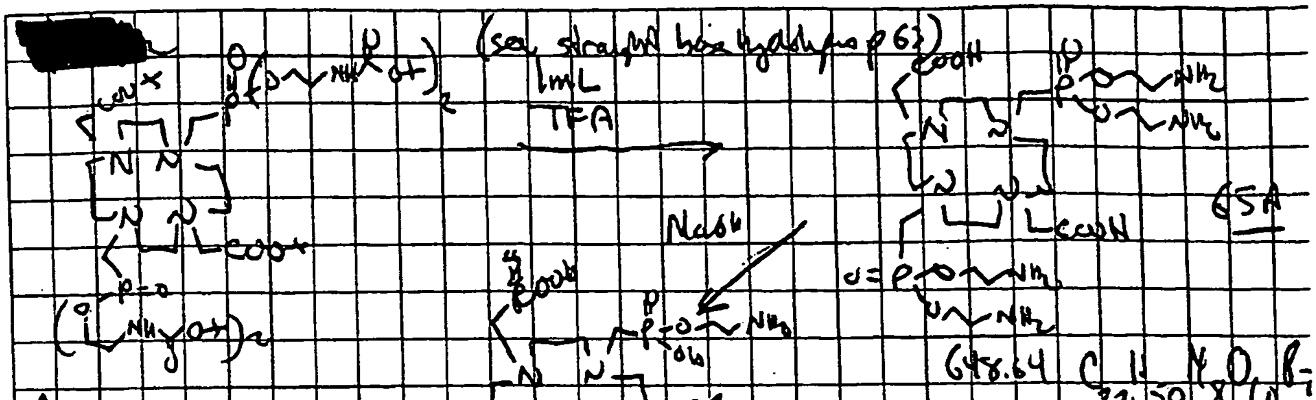
Joseph R. Deven — Signed | [redacted] — Date | Barry Drucker — Signed | [redacted] — Date

PROJECT

Notebook No. A013

Continued From Page –

[redacted]



A006-15
(prep'd A013-31)
24.25 mg = 20.9 µmol

17.4967

→ in glass vial (Tare wt = ~~17.4967~~) dissolve in 1mL TFA start 9pm Thurs 9/26/02
This was slow down w/ argon ~~stre~~ dissolve in 500uL MeOH remove 5uL
+ 400uL MeOH = 65T1 ; Comes out in void volume Run slow gradient
see M+H = 649 & M−H = 647! Looks great. ~~Dissolve in 1mL Methanol~~ Use all in B

(B): All of "A" is dissolved in 500uL of 1N NaOH. leave at room temp
Start 2pm Fri 9/27/02.
check 2pm Sat 9/28/02, Dilute 5uL + 400uL H_2O inject 20uL = 65BT1
comes in void volume see M+H = 606 ~~[illegible]~~ (mono hydrolized)
& M+H = 563 – both weak ; a big 797 ? M+ observed.
Leave ~~to~~ Sitting at room temp ; Monday 9/30 Run dilute 5uL + 400uL H_2O 6[illegible]
looks messy - slightly mostly M+H = 563.
Heat at 63°C Start 2pm 10/1/02 ; Stop 8am 10-4-02. Run 5uL +
400 H_2O, inject 20uL = BT3 Major peaks in void volume = 563 (desired)
and M+23 = 585+, see a slightly retained peak with (+) = 601 a[illegible]
301 which matches for $[M+K^+]^+$ = 562+39 = 601+ & $\frac{602}{2}$ = 301
Gave to Mary to form complex w/ 4

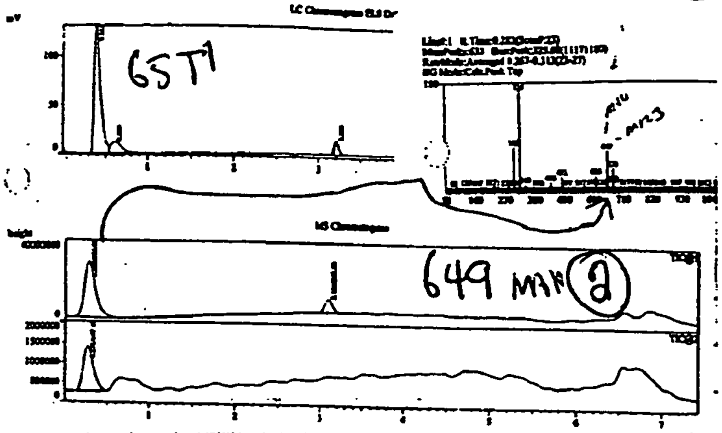
Continued on Page

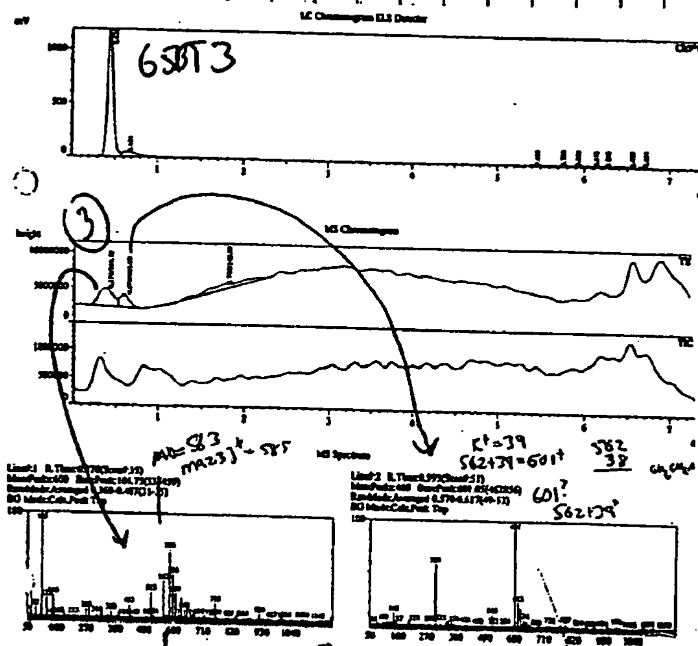
Read and Understood By

Joseph R [illegible] Signed | [redacted] Date | [illegible] Signed | [redacted] Date

Notebook No. N013

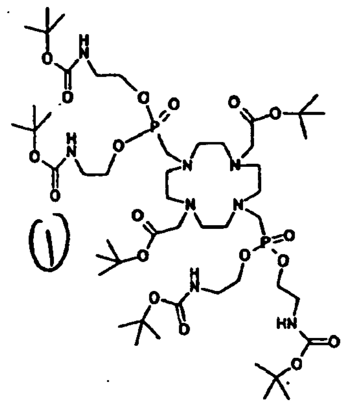
Continued From Page 65





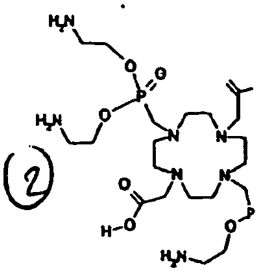
MW=563

A013-65T1



(1)

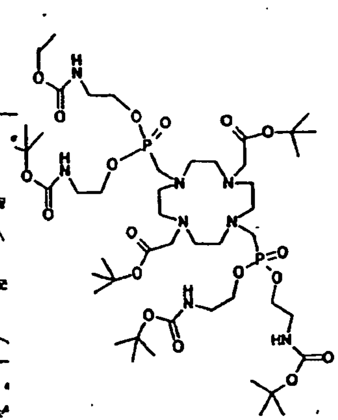
Molecular Weight =1161.33
Exact Mass =1160
Molecular Formula =C50H98N8O18P2



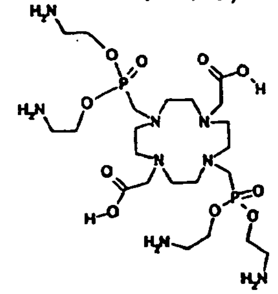
(2)

Molecular Weight =648.64
Exact Mass =648
Molecular Formula =C22H50N8O

A013-65

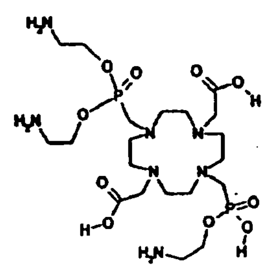


Molecular Weight =1161.33
Exact Mass =1160
Molecular Formula =C50H98N8O18P2



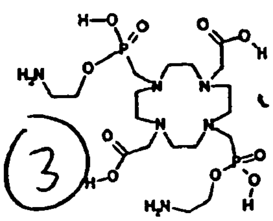
Molecular Weight =648.64
Exact Mass =648
Molecular Formula =C22H50N8O10P2

(B)



Molecular Weight =605.57
Exact Mass =605
Molecular Formula =C20H45N7O10P2

(3)



Molecular Weight =562.50
Exact Mass =562
Molecular Formula =C18H40N6O10P2

Looks like desired product (3) plus K (potassium)

Continued on Page X

Read and Understood By

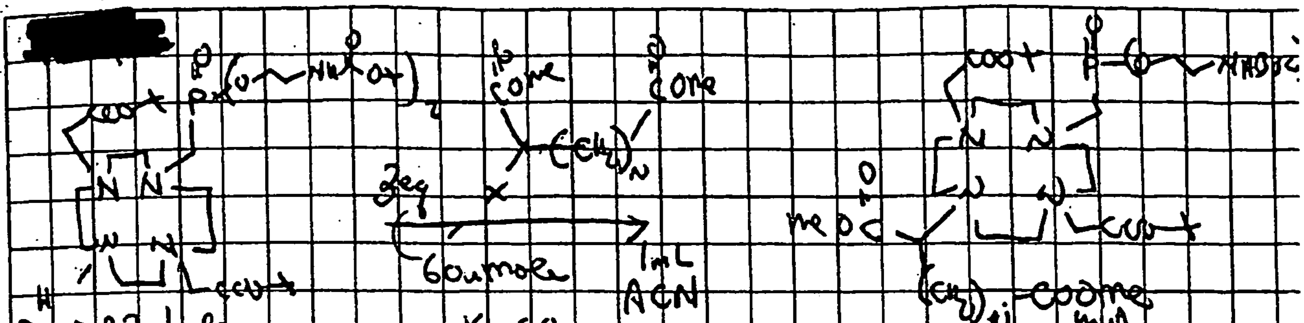
Signed Date [redacted]

Signed Date [redacted]

Notebook No. A013

PROJECT ______ Continued From Page —

[redacted]



780.95 g/mole | K_2CO_3 | MW
Lot A016-15A | 138.21 g/mole | A N=1 925.08
(prep of A013-31 crude) | 10 eq = 200umoles = 28mg | B N=2 939.10
45mg = 57.6 umoles | | C N=3 953.13

÷3 = 15mg each = 19.2 umole ; Pt all reagents in 1mL ACN ; potassium carbonate ~~[illegible]~~ heterogeneously start 9pm Thurs

At 8am (11hrs) sample ~~[illegible]~~ for LCMS: 73A; B; C [illegible] F: 9/27. Very sluggish R

On Sat 2pm add another eqv of alkylating agent & stir Mon 9/30 9pm review - TZ

73A X = OSO_2CF_3 ; Lot A006-4 ; mol wt = 294.20 ; 60 umoles requires 17

N=1

LCMS shows some product (925 M+H) & starting mat M+ = 781 each

add 3ml CH_2Cl_2, filter (0.2u) into seal vial, dry & weigh 13.2536 - 13.2215

Gave to Meg for prep (A016-70; 7.0mg = 32.1mg [illegible]

= ~8 umol ÷ 19.2 = 39.4% yield

73B X = Br ; Lot A009-48B ; mol wt = 239 ; 60umole require 14.3mg

N=2 (added 60mg) (~50uL)

~~All [illegible] Trace of Product - gave to Meg to Prep A016-72~~

DISCARD - NO RX

73C X = Br Lot A015-10 ; mol wt = 253.09 g/mole ; 60umole requires 15mg

N=3 ← some product (m/z=2/2 ~[illegible]) (added 60mg ~50uL)

Only "A" shows any product. B & C show : add 3ml CH_2Cl_2 & filter (0.2

LCMS shows M+2/2 478? dry in Vacuum

slight amount of product 13.7292 - 13.6318 = 97

Gave to Meg to prep (see A016-72 ; she isolated 2.3mg

(this = 2.4 umole ÷ 19.2 umole = 13% isolated yield.

Continued on Page

Read and Understood By

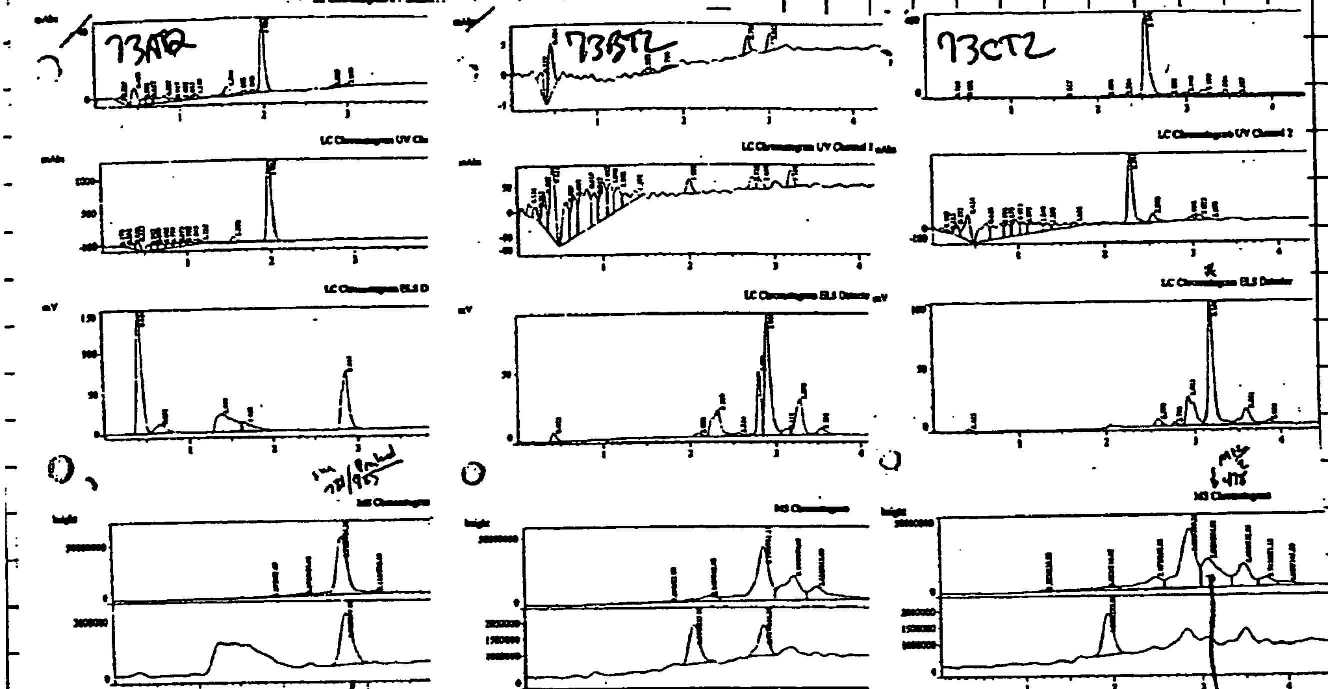
Signed [signature] Date [redacted] Signed [signature] Date [redacted]

PROJECT

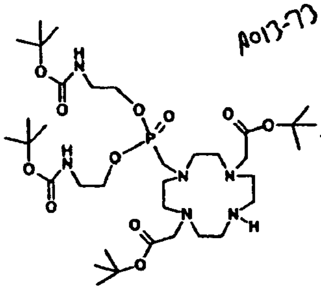
Notebook No. A013

Continued From Page 73

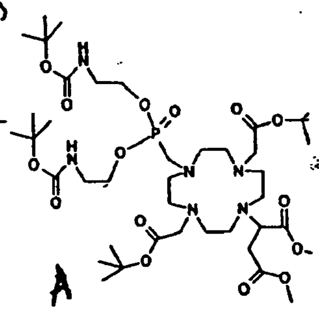
QC HPLC new method A017-XA



A013-73



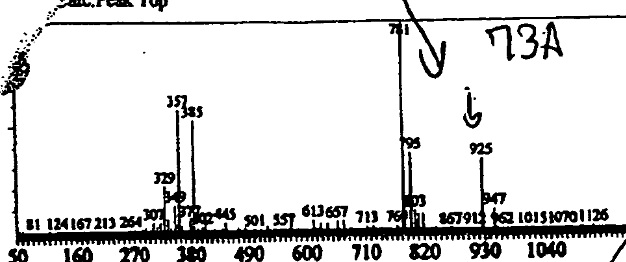
Molecular Weight =780.95
Exact Mass =780
Molecular Formula =C35H69N6O11P



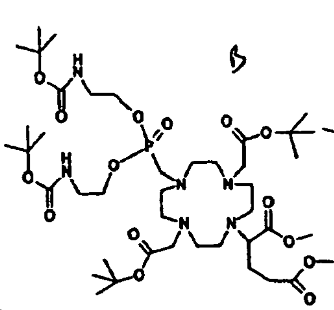
A

Molecular Weight =925.08
Exact Mass =924
Molecular Formula =C41H77N6O15P

2.732(Scan#:233)
BasePeak:781.35(9696904)
Averaged 2.717-2.763(233-237)
Calc.Peak Top

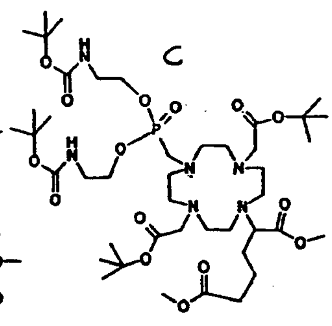


B



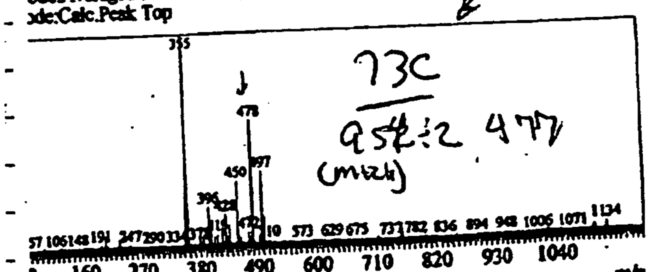
Molecular Weight =939.10
Exact Mass =938
Molecular Formula =C42H79N6O15P

C



Molecular Weight =953.13
Exact Mass =952
Molecular Formula =C43H81N6O15P

R.Time:3.080(Scan#:265)
Peaks:574 BasePeak:354.90(2854823)
Mode:Averaged 3.067-3.113(263-267)
Mode:Calc.Peak Top



Continued on Page X

Read and Understood By

Joseph R. Don — Signed

[redacted] — Date

Barry Drake — Signed

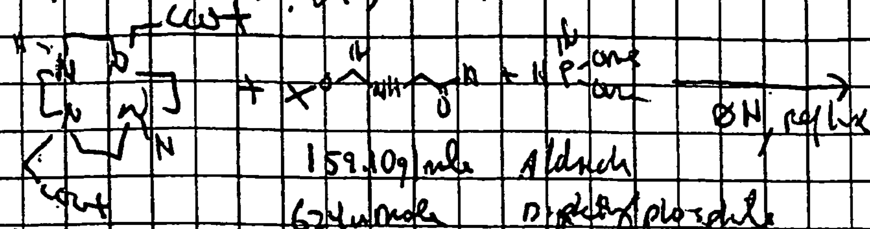
[redacted] — Date

PROJECT

Notebook No. A013

Continued From Page —

[redacted] 1 : 2.5 : 2.2 See similar Rx p31



Mono & Bis ?

400.52 g/mole	159.10 g/mole	Aldrich
100 mg	624 μmole	n-butyl phosphite
250 μmole	99 mg	bp 170°C 110.05 g/mole
		d = 1.20 g/mL
		549 μmole
		60 mg = 50 μL

Mix phosphite + cyclen in 5 mL EtOH & then add the aldehyde
in 1 mL EtOH ; heat at 90°C 3-4 hrs (use timer) Start 3:30 pm. Stop
3:00 pm remove 20 μL & blowdown take up in 40 μL MeOH = 85T
LC-MS trace of mono M+H+ 552 at 2.7 min
add more of aldehyde & phosphite & cook overnight ~12 hrs
Took ~1 mg of Solid aldehyde + 4 μL MeOH = 855 MA
Let set until 5 pm 10-15-02. remove 20 μL & blowdown, take up in
40 μL MeOH = 85T2 Very small amount of the ~~bis~~ mono product &
No Bis product.

Continued on Page

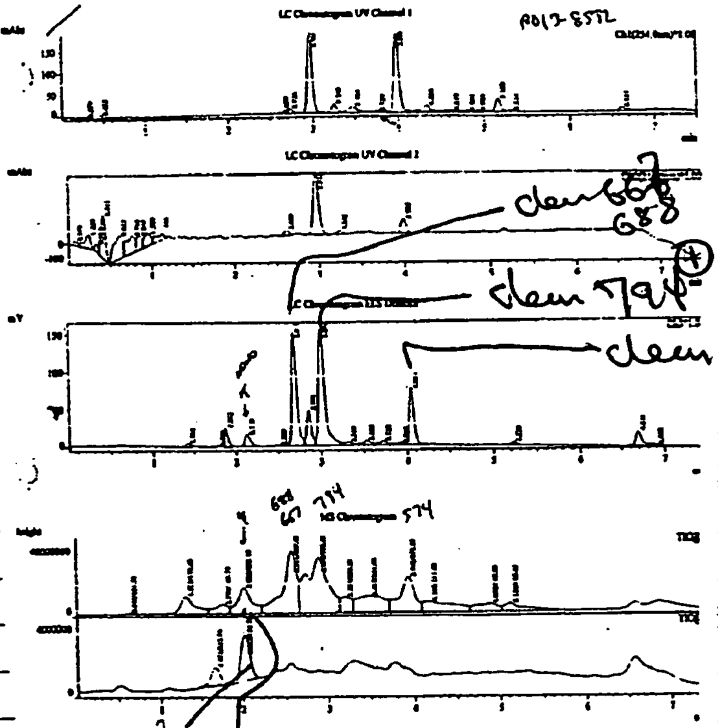
Read and Understood By

Signed [redacted] Date Signed [redacted] Date

PROJECT

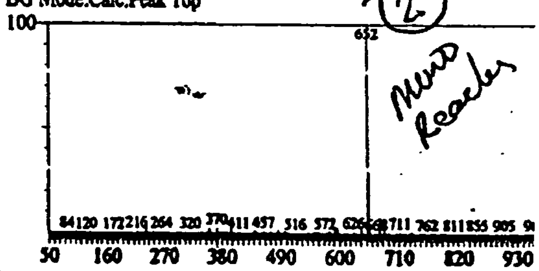
Notebook No. A013
Continued From Page 85

Crude = A013-1A



UV peaks gone at T2.

Line#:4 R.Time:2.025(Scan#:173)
MassPeaks:550 BasePeak:652.10(6834055)
RawMode:Averaged 1.993-2.040(171-175)
BG Mode:Calc.Peak Top

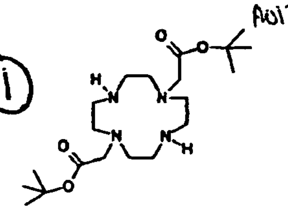


(2)

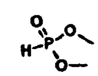
Mono Reacts

A013-85

(1)

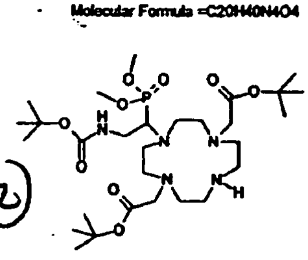


Molecular Weight =400.57
Exact Mass =400
Molecular Formula =C20H40N4O4



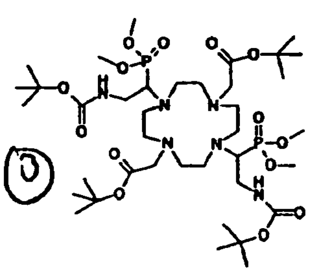
Molecular Weight =110.05
Exact Mass =110
Molecular Formula =C2H7O3P

(2)



Molecular Weight =651.79
Exact Mass =651
Molecular Formula =C29H58N5O9P

(3)



Molecular Weight =903.01
Exact Mass =902
Molecular Formula =C38H76N6O14P2

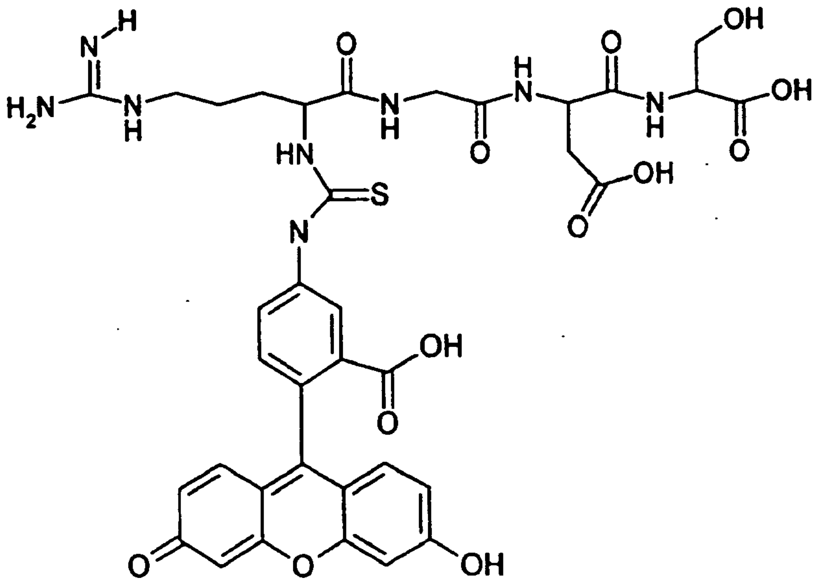
Rt time 2.126 min
Mono MW 652

Continued on Page

Read and Understood By

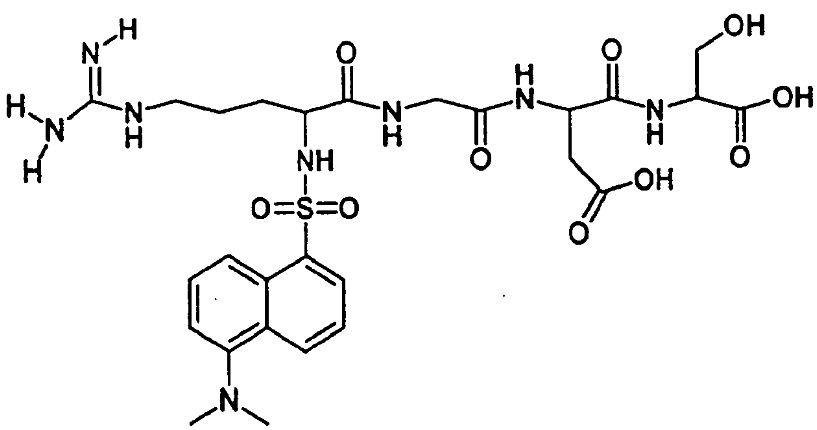
Signed [redacted] Date Signed [redacted] Date

750 uL of Solution of 1mMolar Ligand in PBS; lot= A023-51A



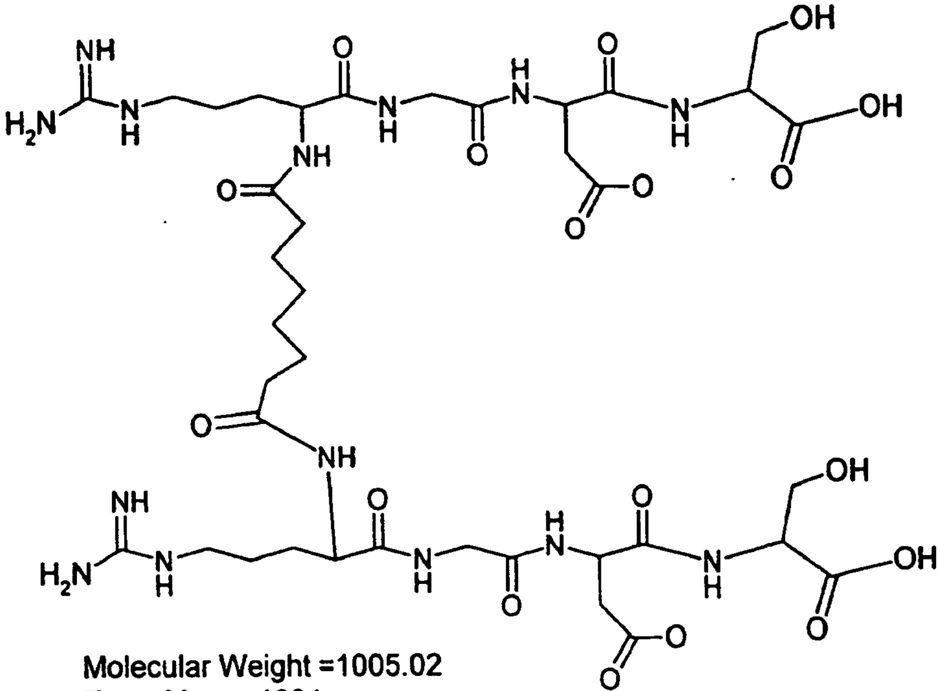
Molecular Weight =822.81
Exact Mass =822
Molecular Formula =C36H38N8O13S

1 mL of Solution of 1mMolar Ligand in PBS; lot= A023-51B



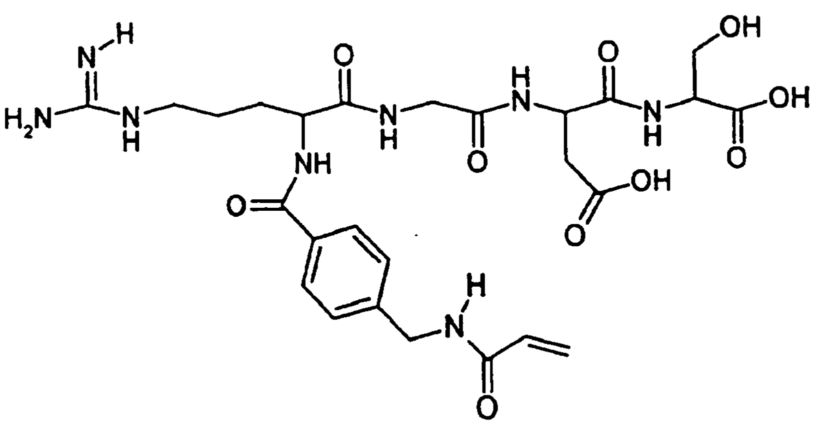
Molecular Weight =666.72
Exact Mass =666
Molecular Formula =C27H38N8O10SS

500 uL of Solution of 1mMolar Ligand in PBS; lot= A023-51C



Molecular Weight =1005.02
Exact Mass =1004
Molecular Formula =C38H64N14O18

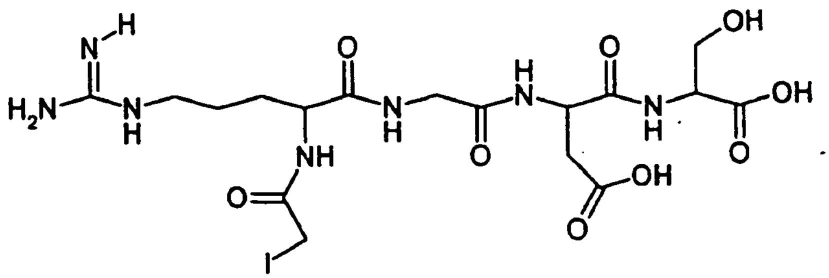
1 mL of Solution of 1mMolar Ligand in PBS; lot= A023-51D



Molecular Weight =620.62
Exact Mass =620
Molecular Formula =C26H36N8O10

1 mL of Solution of 1mMolar Ligand (lot A023-27B) in PBS; lot= A023-52A

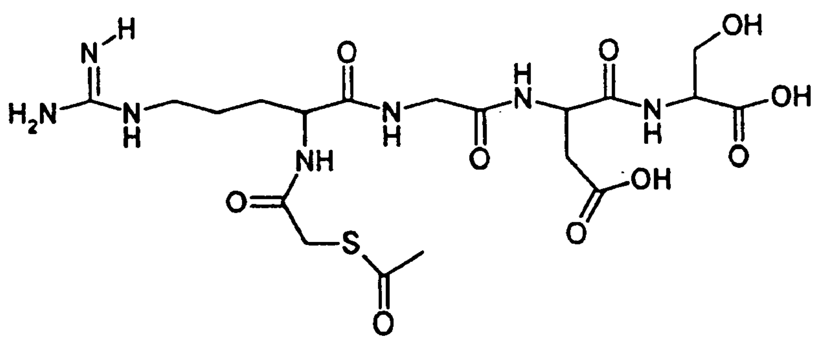
1 mL of Solution of 1mMolar Ligand (lot A023-27) in PBS; lot= A023-52B



Molecular Weight =601.36
Exact Mass =601
Molecular Formula =C17H28IN7O9

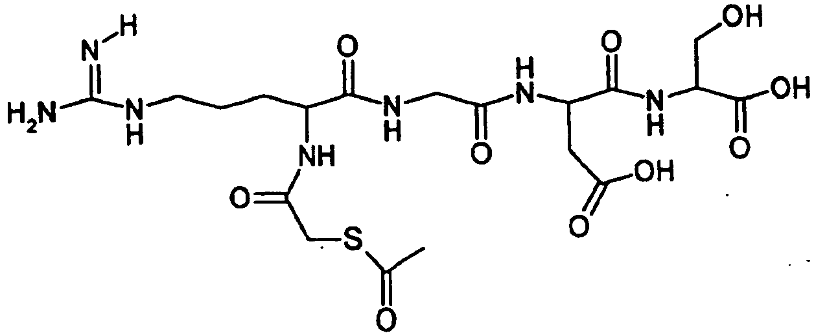
500 uL of Solution of 1mMolar Ligand (lot A023-37B) in PBS; lot= A023-52C

1 mL of Solution of 1mMolar Ligand (lot A023-19) in PBS; lot= A023-52D



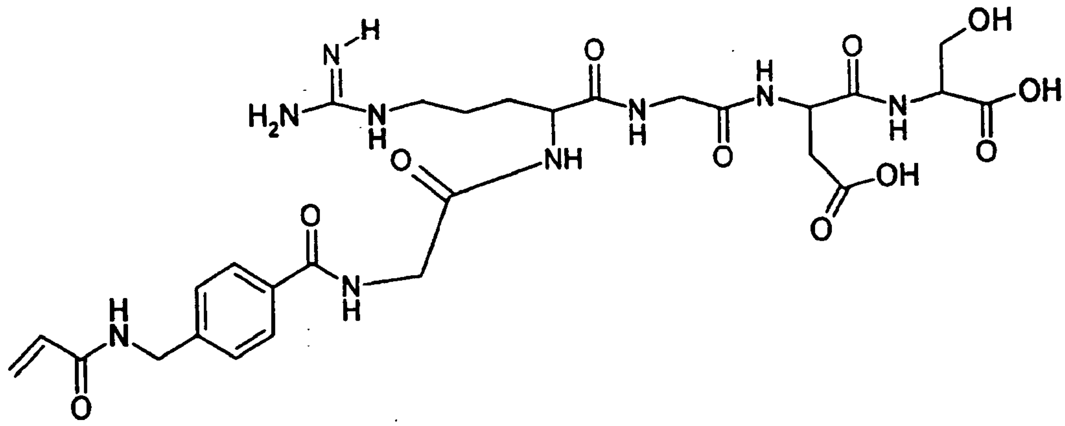
Molecular Weight =549.56
Exact Mass =549
Molecular Formula =C19H31N7O10S

500 uL of Solution of 1mMolar Ligand (lot A023-19B) in PBS; lot= A023-53A



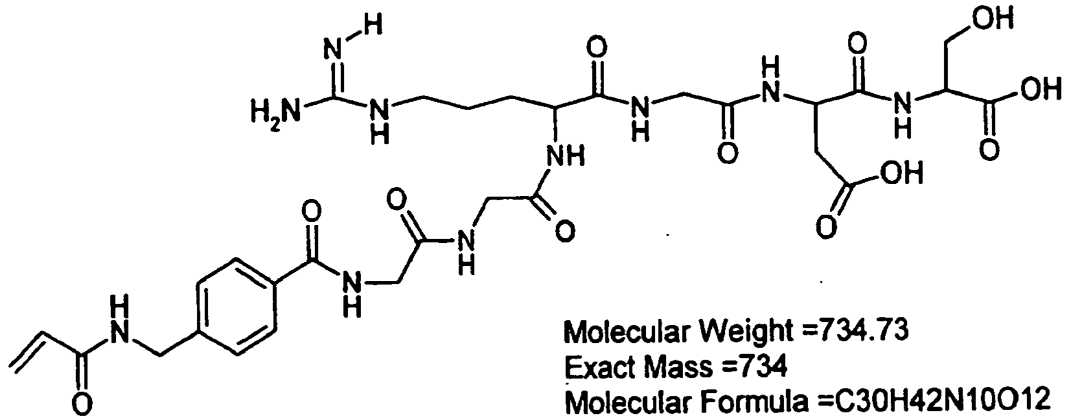
Molecular Weight =549.56
Exact Mass =549
Molecular Formula =C19H31N7O10S

1.474 mL of Solution of 1mMolar Ligand (lot A023-61) in PBS; lot= A024-93A

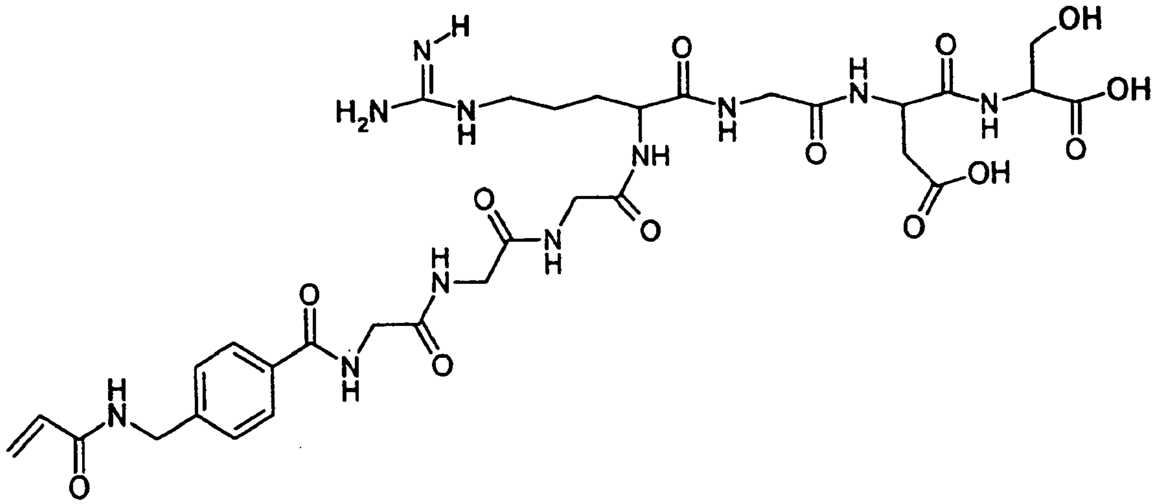


Molecular Weight =677.68
Exact Mass =677
Molecular Formula =C28H39N9O11

2.042 mL of Solution of 1mMolar Ligand (lot A023-65B) in PBS; lot= A024-93B

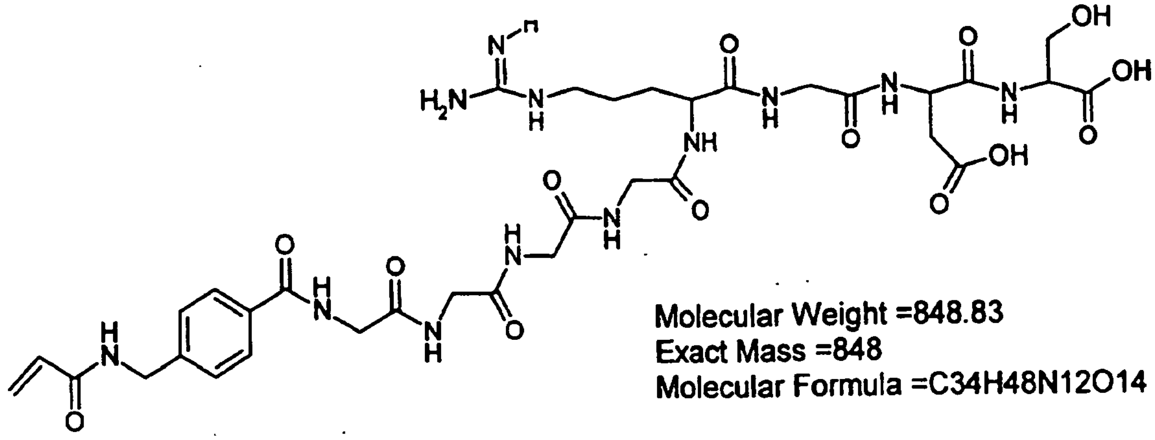


Molecular Weight =734.73
Exact Mass =734
Molecular Formula =C30H42N10O12



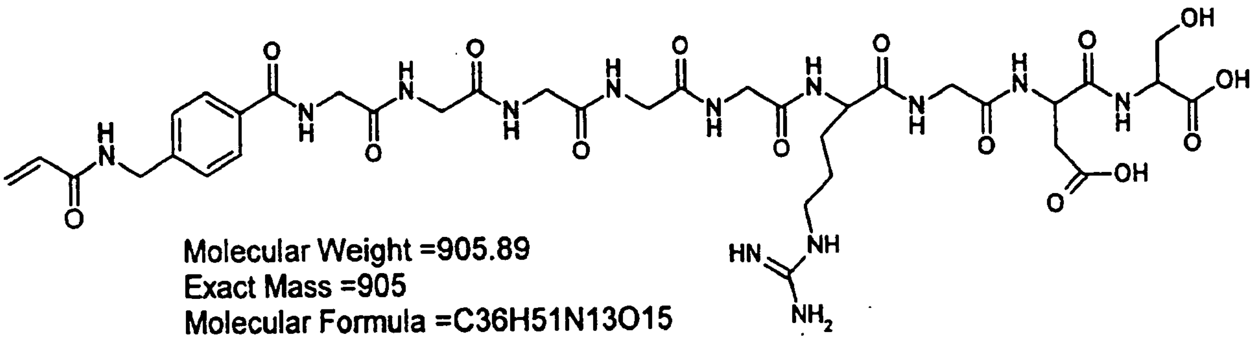
Molecular Weight =791.78
Exact Mass =791
Molecular Formula =C32H45N11O13

589 uL of Solution of 1mMolar Ligand (lot A023-69A) in PBS; lot= A024-93D



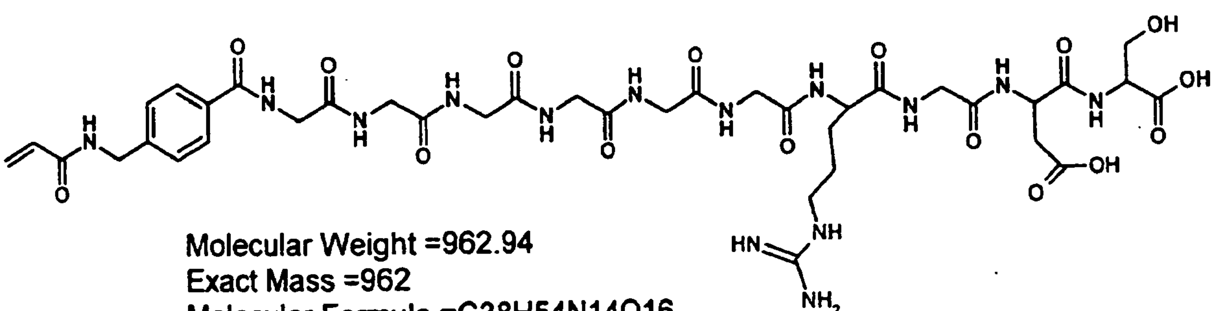
Molecular Weight =848.83
Exact Mass =848
Molecular Formula =C34H48N12O14

1.014 mL of Solution of 1mMolar Ligand (lot A023-71A) in PBS; lot= A024-93E



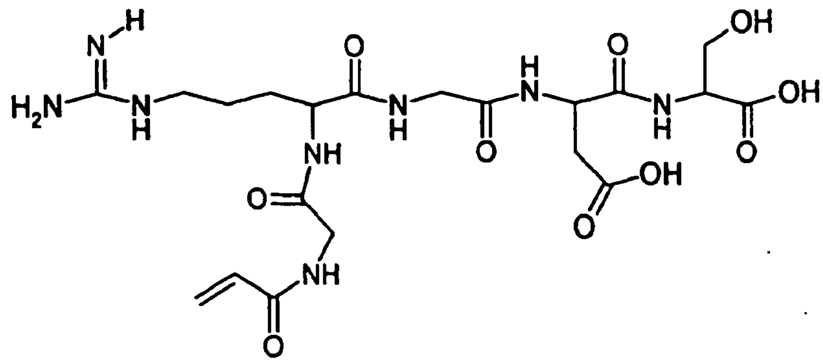
Molecular Weight =905.89
Exact Mass =905
Molecular Formula =C36H51N13O15

1.142 mL of Solution of 1mMolar Ligand (lot A023-73A) in PBS; lot= A024-93F



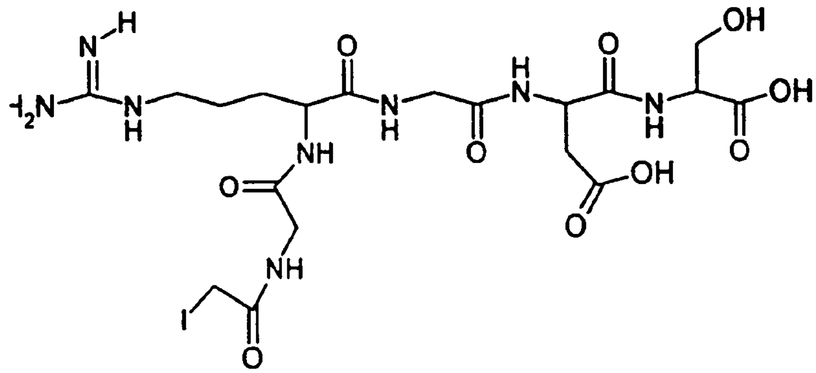
Molecular Weight =962.94
Exact Mass =962
Molecular Formula =C38H54N14O16

2.390 mL of Solution of 1mMolar Ligand (lot A023-88A) in PBS; lot= A027-3A



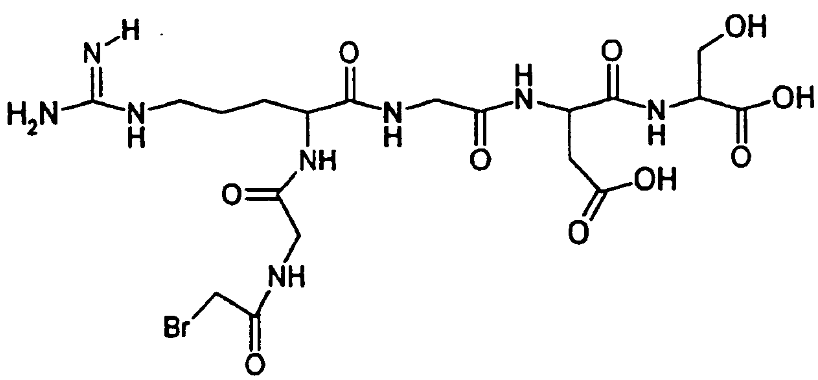
Molecular Weight =544.53
Exact Mass =544
Molecular Formula =C20H32N8O10

0.760 mL of Solution of 1mMolar Ligand (lot A023-86B) in PBS; lot= A027-3B



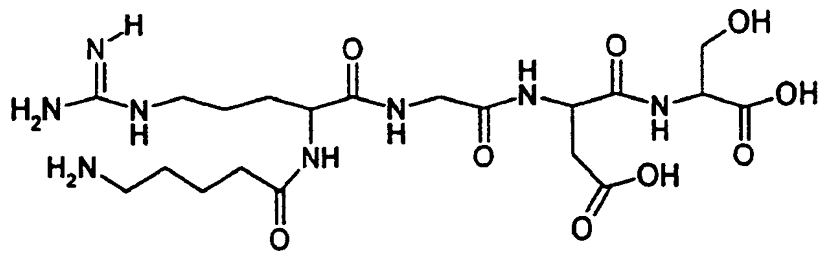
Molecular Weight =658.41
Exact Mass =658
Molecular Formula =C19H31IN8O10

0.820 mL of Solution of 1mMolar Ligand (lot A023-84B) in PBS; lot= A027-3C



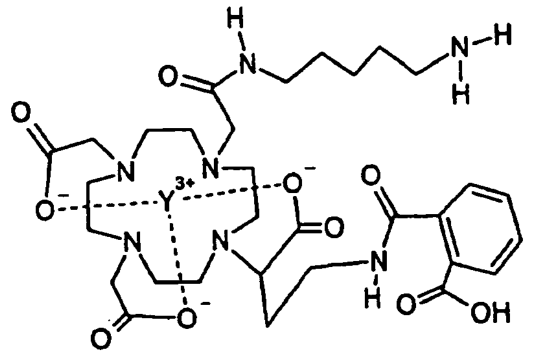
Molecular Weight =611.41
Exact Mass =611
Molecular Formula =C19H31BrN8O10

2.065 mL of Solution of 1mMolar Ligand (lot A023-90) in PBS; lot= A027-3D



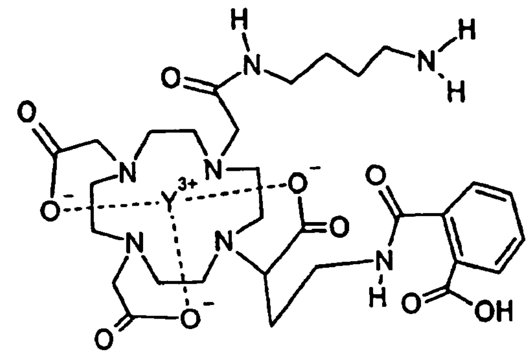
Molecular Weight =532.56
Exact Mass =532
Molecular Formula =C20H36N8O9

Solution of 1 mMolar Complex in PBS = A012-56A
Ligand= lot A012-17
Complex= A012-32



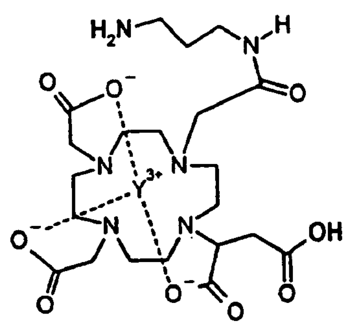
Molecular Weight =765.66
Exact Mass =765
Molecular Formula =C31H46N7O10Y

Solution of 1mMolar Complex in PBS= A012-56B
Ligand = lot A012-19
Complex= lot A012-37



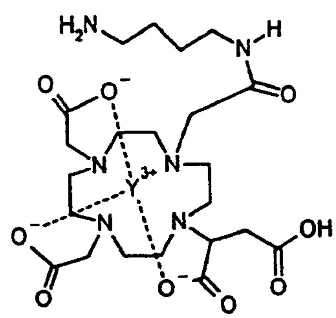
Molecular Weight =751.63
Exact Mass =751
Molecular Formula =C30H44N7O10Y

Solution of 1mMolar Complex in PBS= A012-56C
Ligand = lot A011-75B
Complex= lot A012-44



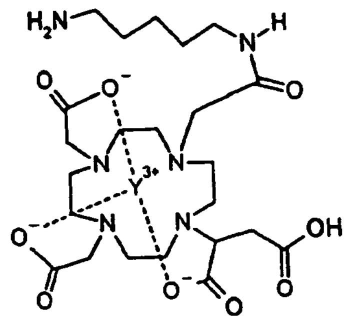
Molecular Weight =604.45
Exact Mass =604
Molecular Formula =C21H35N6O9Y

Solution of 1mMolar Complex in PBS= A012-56D
Ligand= lot A011-75C
Complex = lot A012-46



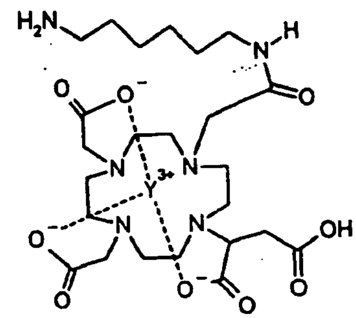
Molecular Weight =618.48
Exact Mass =618
Molecular Formula =C22H37N6O9Y

Solution of 1mMolar Complex in PBS= A012-57A
Ligand = lot A011-75D
Complex= lot A012-48



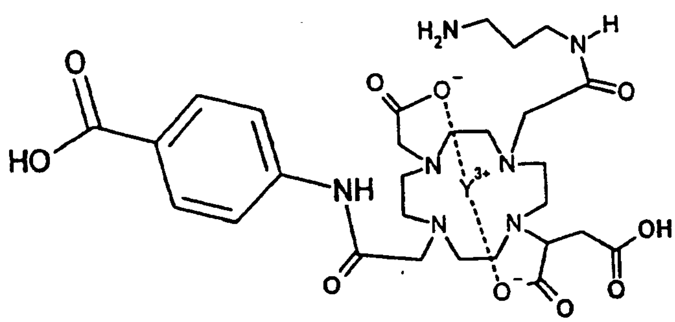
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS= A012-57B
Ligand= lot A011-75E
Complex = lot A012-50



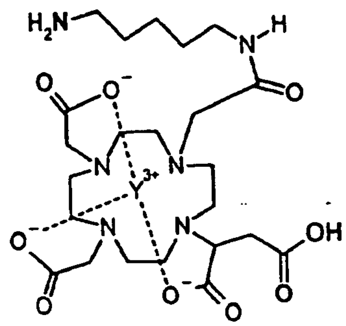
Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS= A012-57C
Ligand = lot A011-65C
Complex= lot A012-25



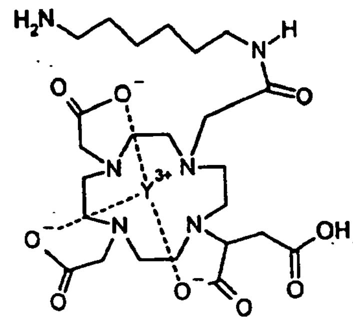
Molecular Weight =724.58
Exact Mass =724
Molecular Formula =C28H41N7O10Y

Solution of 1mMolar Complex in PBS= A012-57A
Ligand = lot A011-75D
Complex= lot A012-48



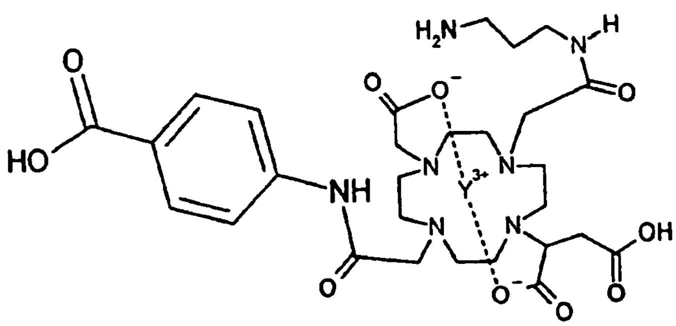
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS= A012-57B
Ligand= lot A011-75E
Complex = lot A012-50



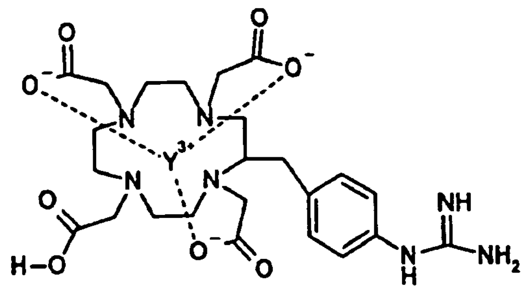
Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS= A012-57C
Ligand = lot A011-65C
Complex= lot A012-25



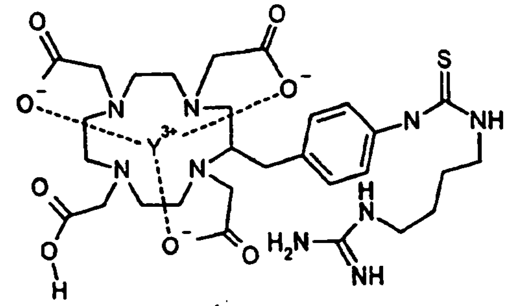
Molecular Weight =724.58
Exact Mass =724
Molecular Formula =C28H41N7O10Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80A PBS



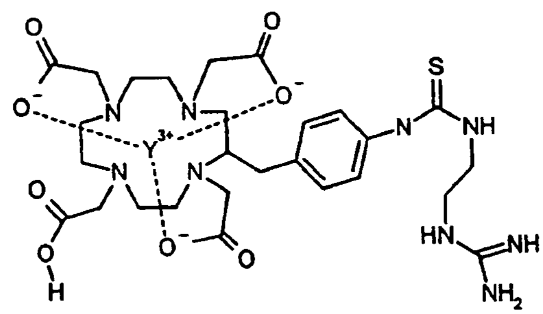
Molecular Weight =637.49
Exact Mass =637
Molecular Formula =C24H34N7O8Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80B PBS



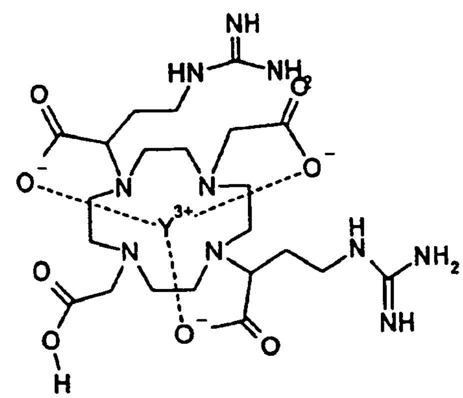
Molecular Weight =767.70
Exact Mass =767
Molecular Formula =C29H44N9O8SY

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80C PBS



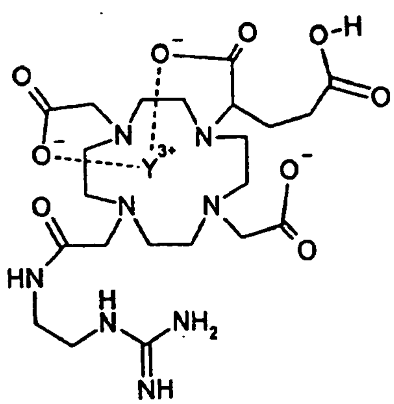
Molecular Weight =739.64
Exact Mass =739
Molecular Formula =C27H40N9O8SY

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80D PBS



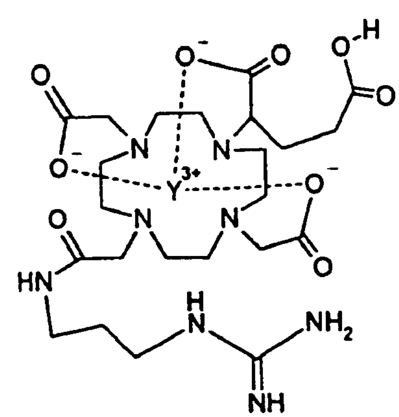
Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C22H39N10O8Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80E PBS



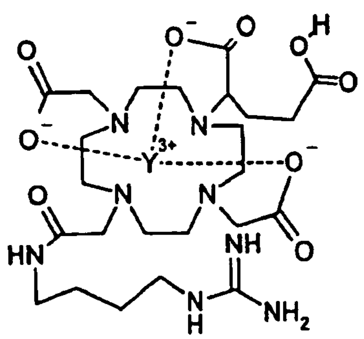
Molecular Weight =646.49
Exact Mass =646
Molecular Formula =C22H37N8O9Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80F PBS



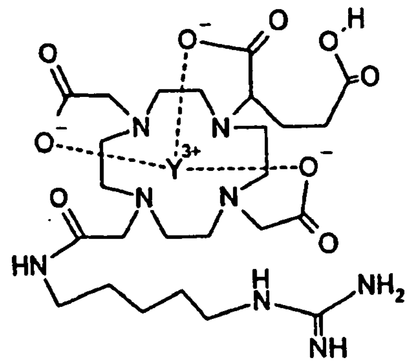
Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C23H39N8O9Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80G PBS



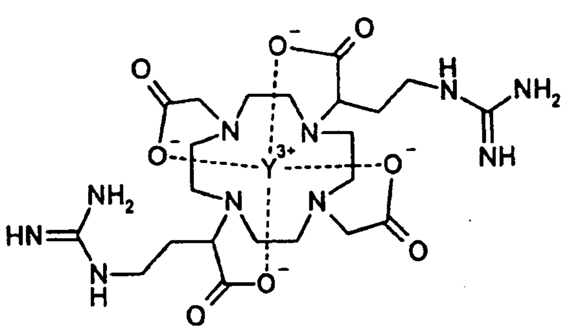
Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80H PBS



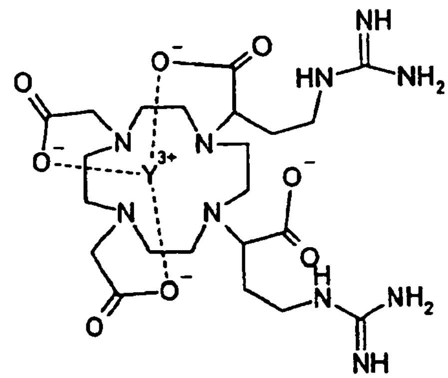
Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80I PBS



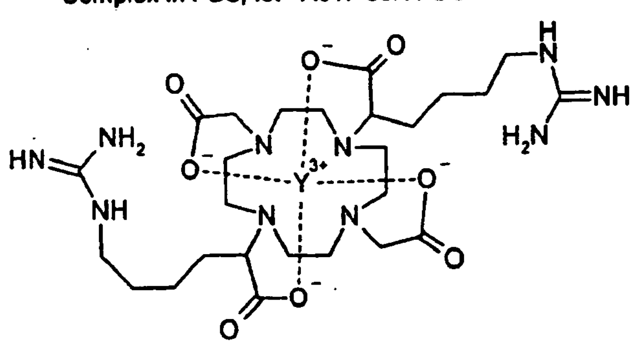
Molecular Weight =659.52
Exact Mass =659
Molecular Formula =C22H38N10O8Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80J PBS



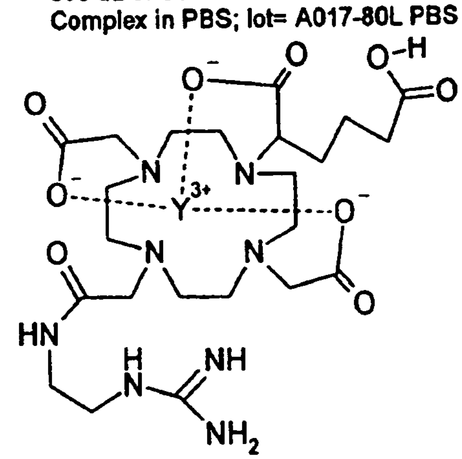
Molecular Weight =659.52
Exact Mass =659
Molecular Formula =C22H38N10O8Y

500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80K PBS



Molecular Weight =715.62
Exact Mass =715
Molecular Formula =C26H46N10O8Y

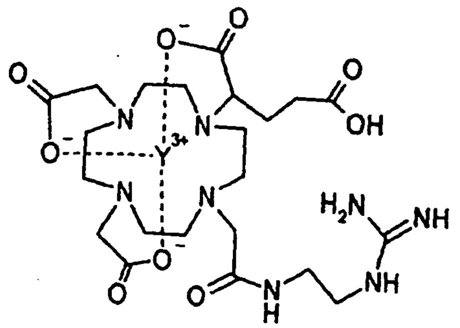
500 uL of Solution of 1mMolar
Complex in PBS; lot= A017-80L PBS



Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C23H39N8O9Y

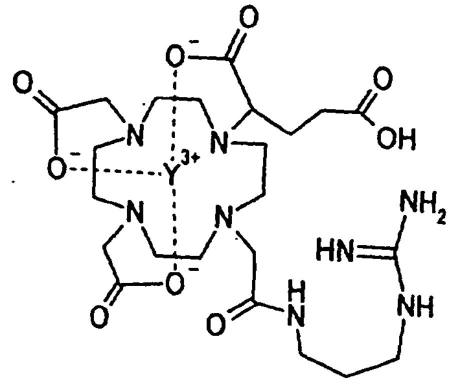
500 uL of Solution of 1mMolar
Blank mixture in PBS; lot= A017-80Z PBS

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16Q



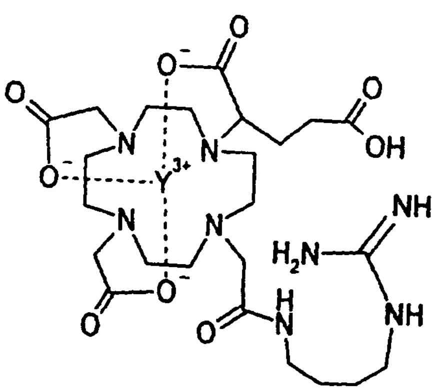
Molecular Weight =646.49
Exact Mass =646
Molecular Formula =C22H37N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16R



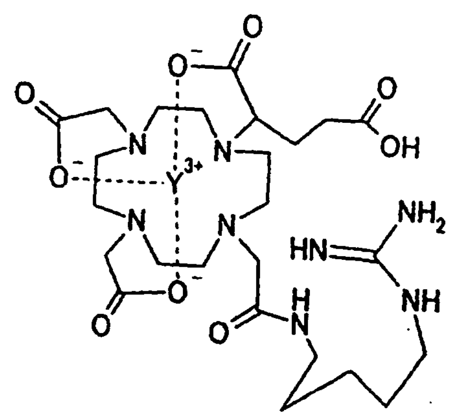
Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C23H39N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16S



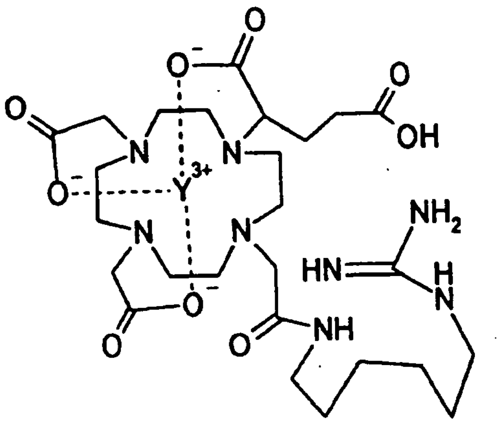
Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

185 uL of 1 mMolar Complex in PBS
Lot Number= A024-16T



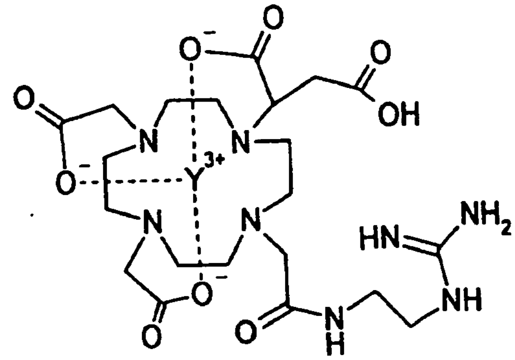
Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

260 uL of 1 mMolar Complex in PBS
Lot Number= A024-16U



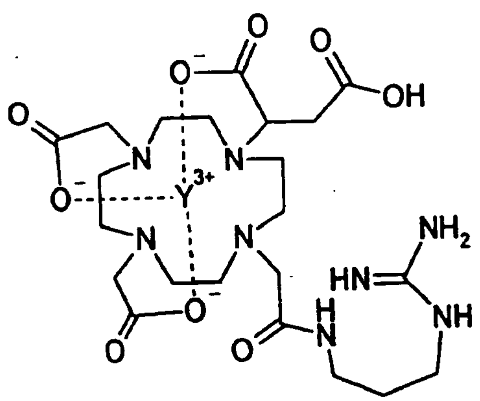
Molecular Weight =702.60
Exact Mass =702
Molecular Formula =C26H45N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16V



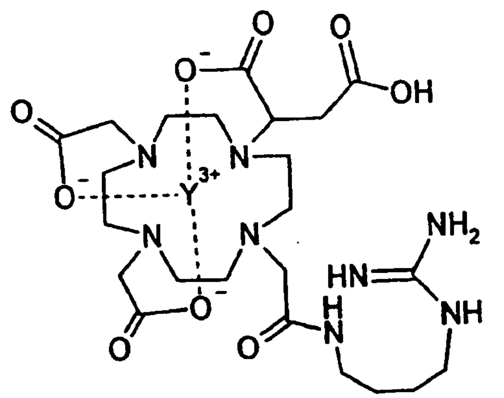
Molecular Weight =632.47
Exact Mass =632
Molecular Formula =C21H35N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16W



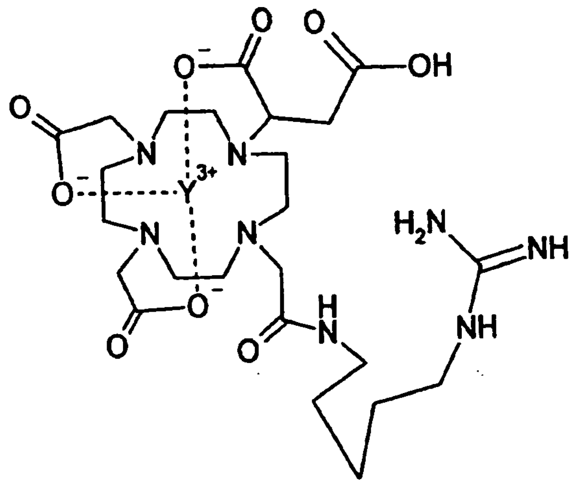
Molecular Weight =646.49
Exact Mass =646
Molecular Formula =C22H37N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16X



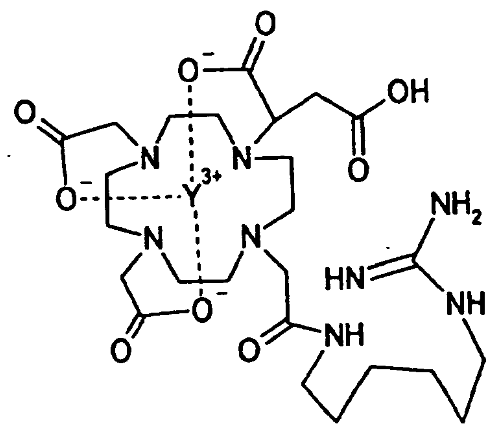
Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C23H39N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16Y



Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

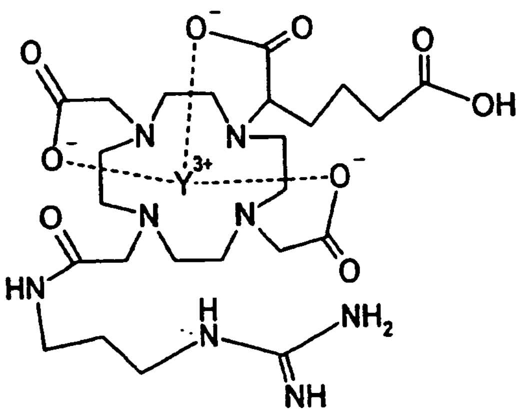
500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16Z



Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

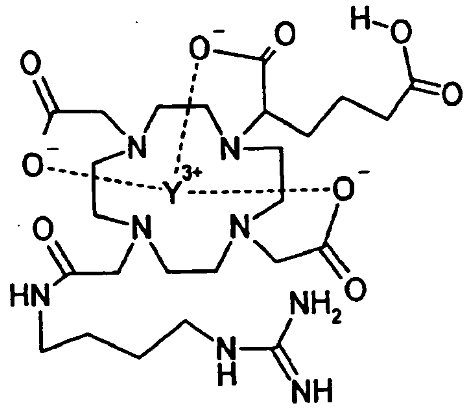
500 uL of 1 mMolar Blank Reaction Mixture in PBS
Lot Number= A024-16BLANK

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16M



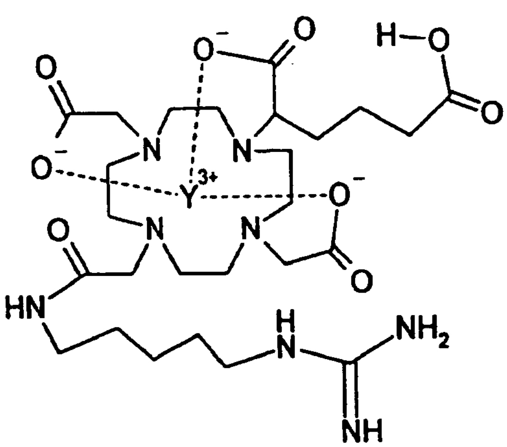
Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16N



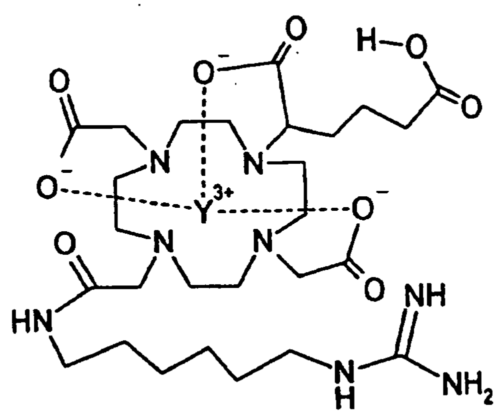
Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16O



Molecular Weight =702.60
Exact Mass =702
Molecular Formula =C26H45N8O9Y

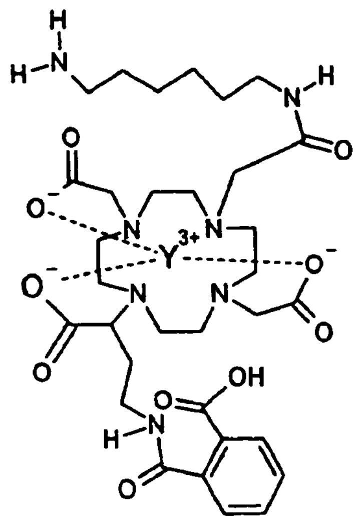
500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16P



Molecular Weight =716.63
Exact Mass =716
Molecular Formula =C27H47N8O9Y

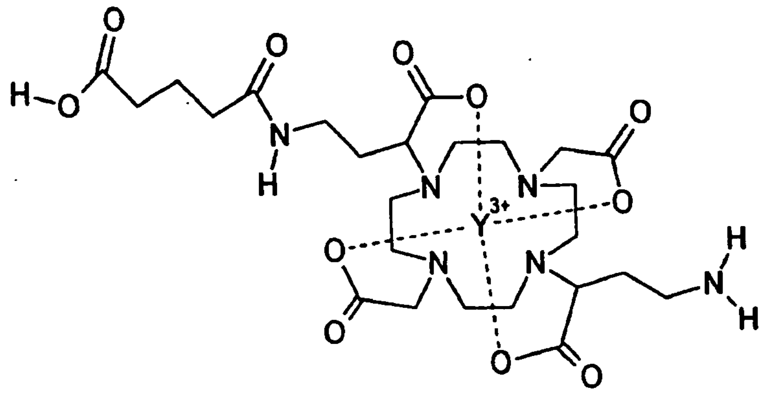
Complexes for Purdue [redacted]

Solution of 1mMolar Complex in PBS= A007-96A
Ligand= lot A007-77(prep of A011-21)
Complex= lot A007-91



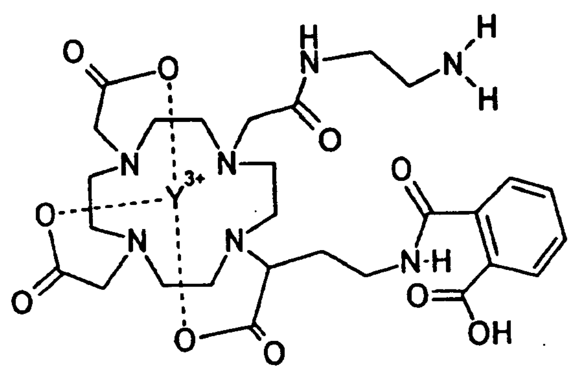
Molecular Weight =779.69
Exact Mass =779
Molecular Formula =C32H48N7O10Y

Solution of 1mMolar Complex in PBS= A007-96B
Ligand= lot A011-25
Complex= lot A007-92

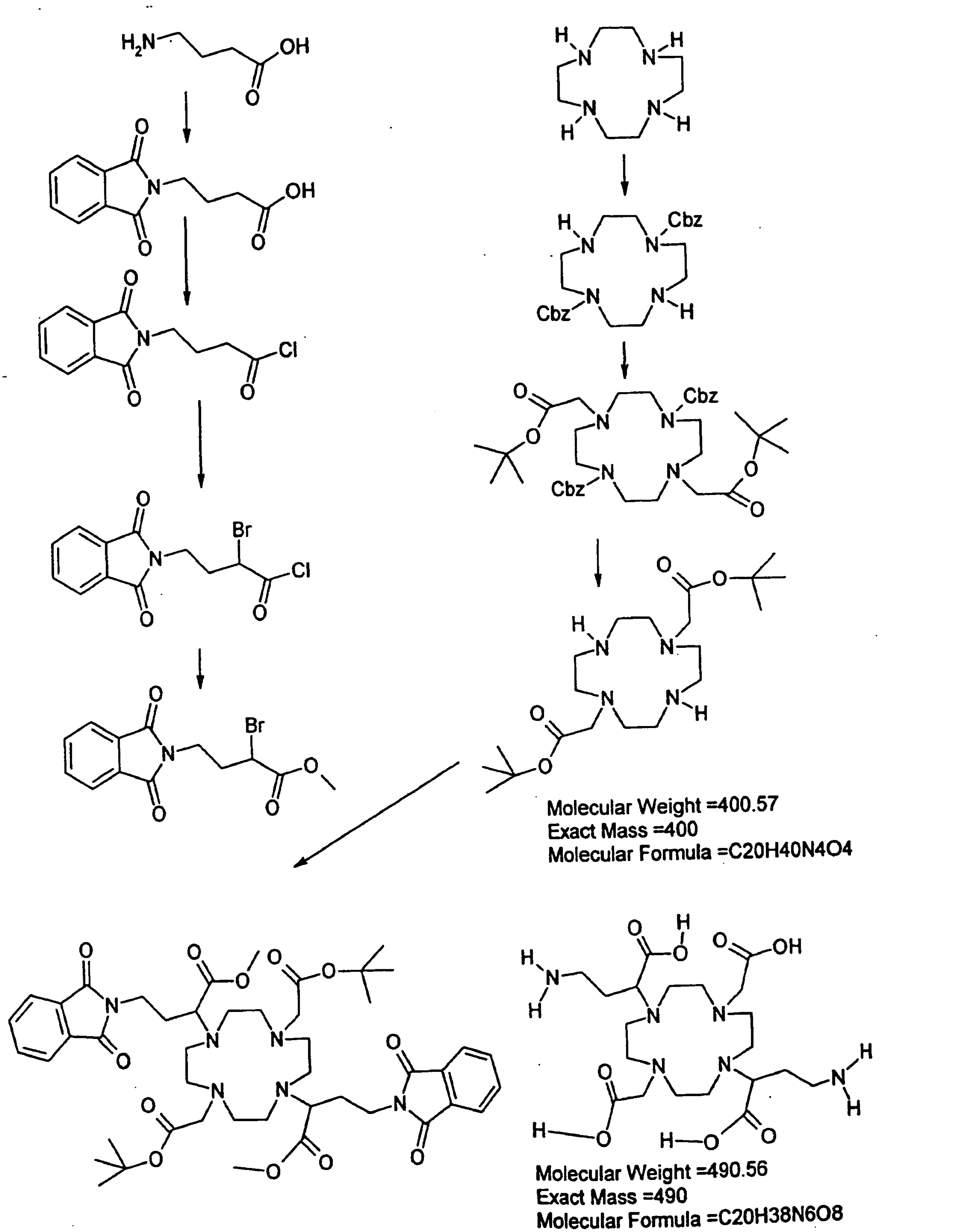


Molecular Weight =689.54
Exact Mass =689
Molecular Formula =C25H40N6O11Y

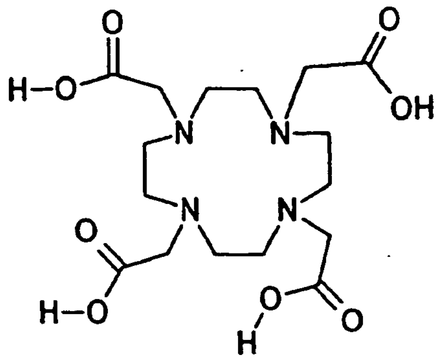
Solution of 1mMolar Complex in PBS= A007-96C
Ligand= lot A007-89 (prep of A011-33)
Complex= lot A007-93



Molecular Weight =723.58
Exact Mass =723
Molecular Formula =C28H40N7O10Y

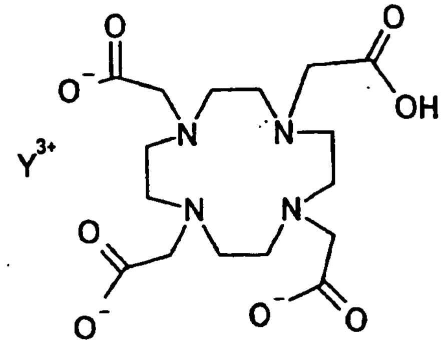

H2N
OH
O
Cl
Br
Cbz
H
N
Molecular Weight =400.57
Exact Mass =400
Molecular Formula =C20H40N4O4
Molecular Weight =490.56
Exact Mass =490
Molecular Formula =C20H38N6O8

DOTA

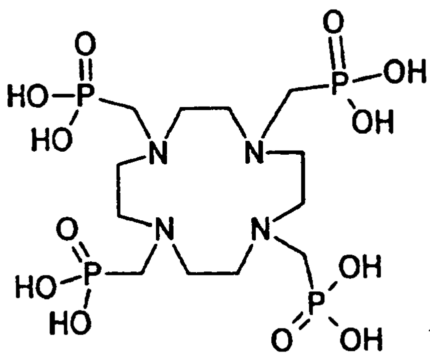


Molecular Weight =404.42
Exact Mass =404
Molecular Formula =C16H28N4O8

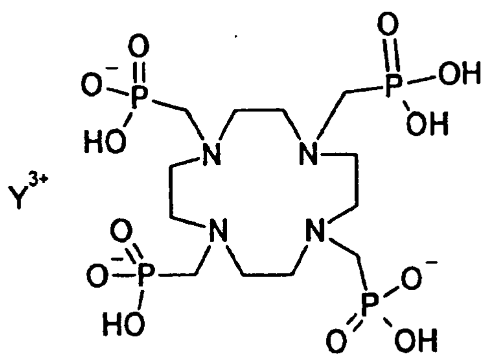
Y-DOTA



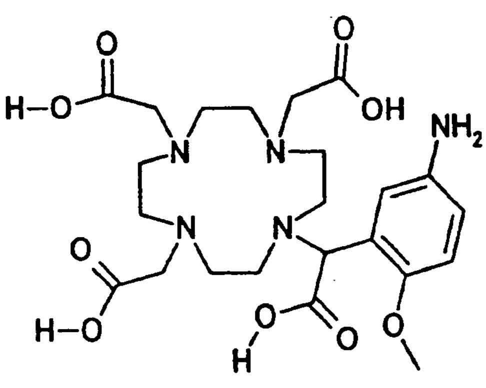
Molecular Weight =401.40 88.91
Exact Mass =401 89
Molecular Formula =C16H25N4O8 . Y



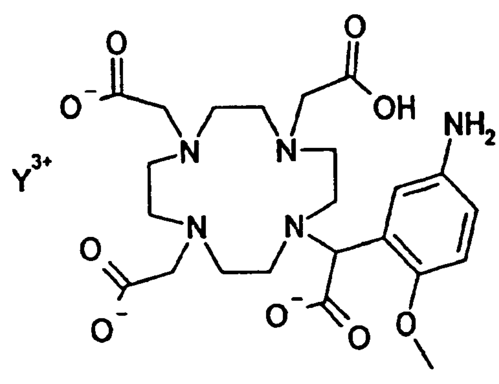
Molecular Weight =548.30
Exact Mass =548
Molecular Formula =C12H32N4O12P4



Molecular Weight =545.28 88.91
Exact Mass =545 89
Molecular Formula =C12H29N4O12P4 . Y

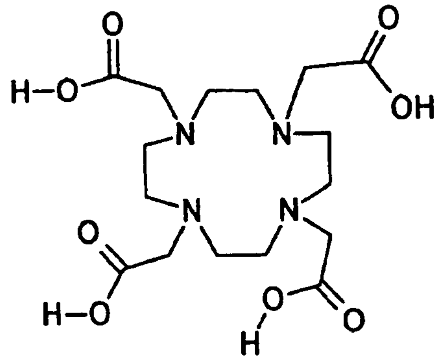


Molecular Weight =525.56
Exact Mass =525
Molecular Formula =C23H35N5O9



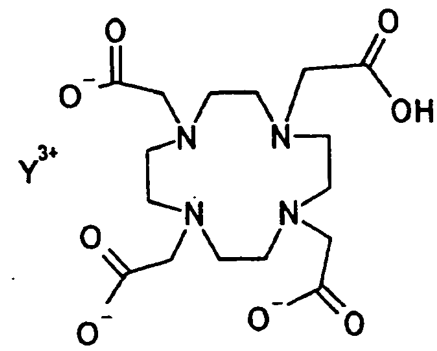
Molecular Weight =522.54 88.91
Exact Mass =522 89
Molecular Formula =C23H32N5O9 . Y

DOTA

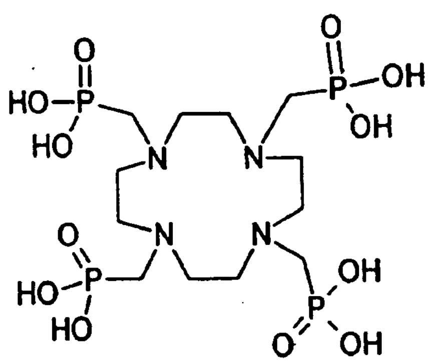


Molecular Weight =404.42
Exact Mass =404
Molecular Formula =C16H28N4O8

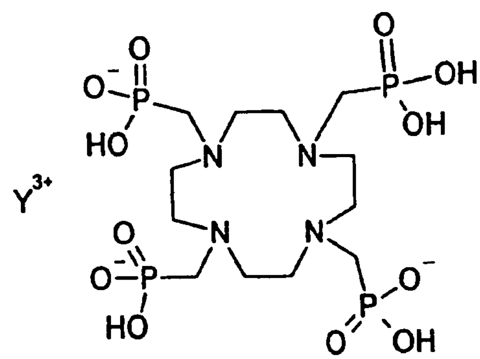
Y-DOTA



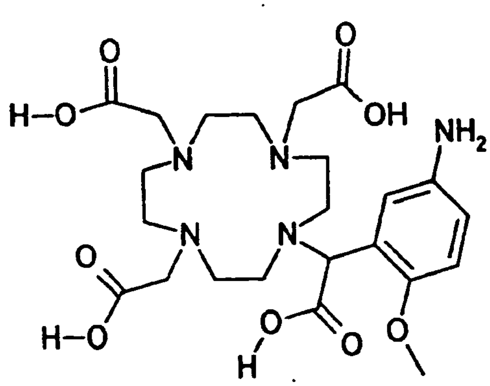
Molecular Weight =401.40 88.91
Exact Mass =401 89
Molecular Formula =C16H25N4O8 . Y



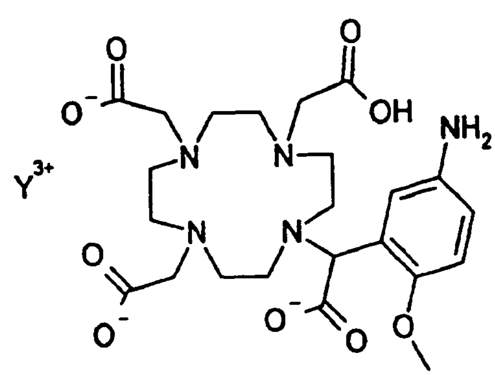
Molecular Weight =548.30
Exact Mass =548
Molecular Formula =C12H32N4O12P4



Molecular Weight =545.28 88.91
Exact Mass =545 89
Molecular Formula =C12H29N4O12P4 . Y

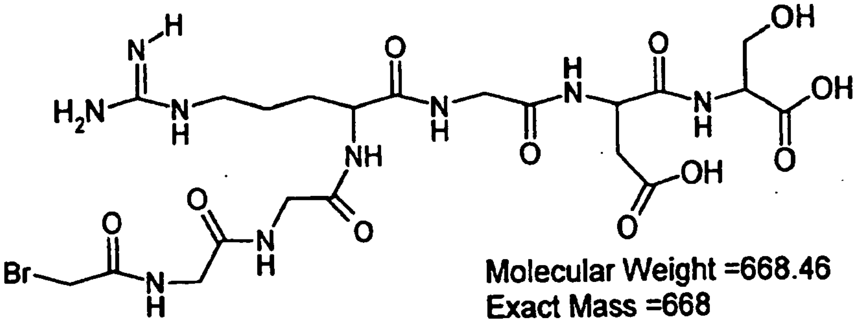


Molecular Weight =525.56
Exact Mass =525
Molecular Formula =C23H35N5O9



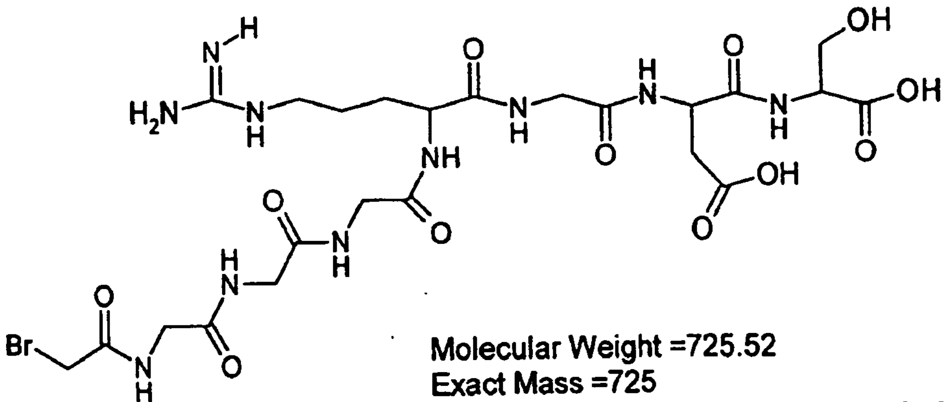
Molecular Weight =522.54 88.91
Exact Mass =522 89
Molecular Formula =C23H32N5O9 . Y

0.5 mL of Solution of 1 mMolar Ligand (lot A023-96)
in PBS, Lot=A028-21A; pH=7.43



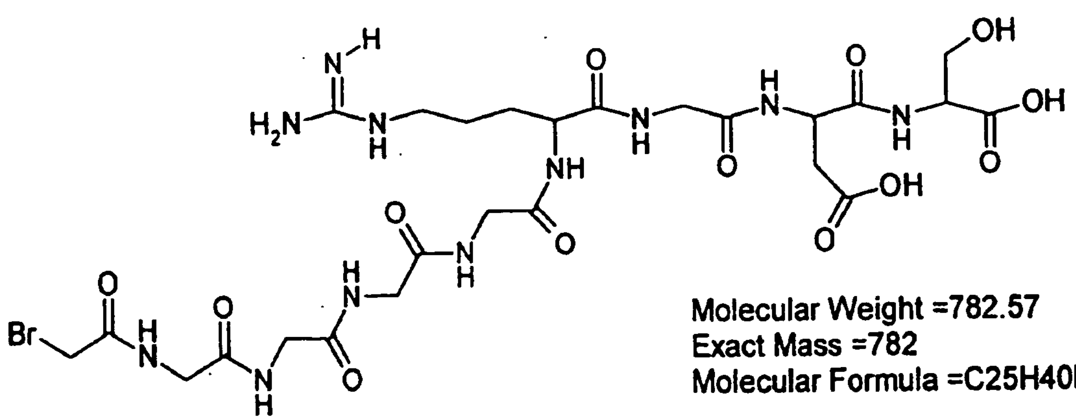
Molecular Weight =668.46
Exact Mass =668
Molecular Formula =C21H34BrN9O11

0.875 mL of Solution of 1 mMolar Ligand (lot A023-98)
in PBS, Lot=A028-21B; pH=7.39



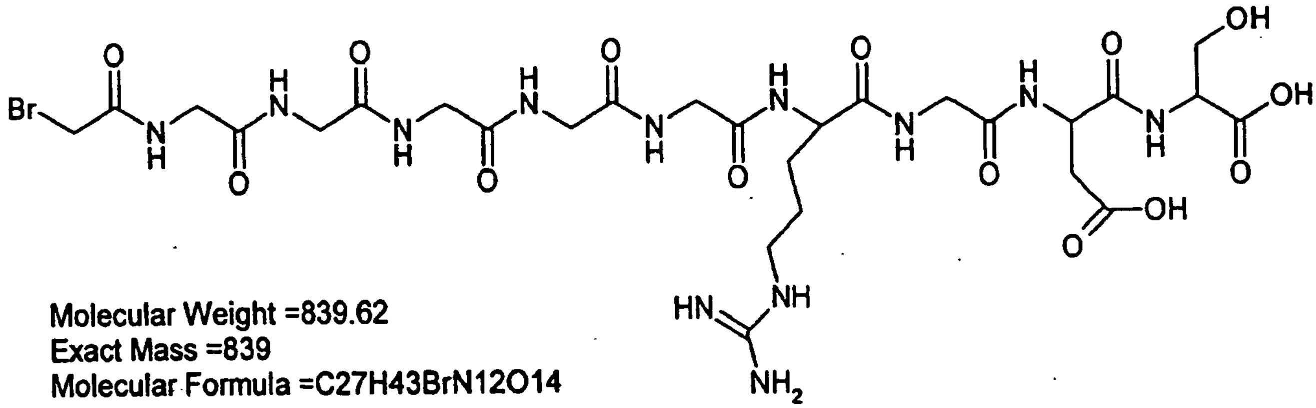
Molecular Weight =725.52
Exact Mass =725
Molecular Formula =C23H37BrN10O12

0.75 mL of Solution of 1 mMolar Ligand (lot A028-01)
in PBS, Lot=A028-21C; pH=7.45



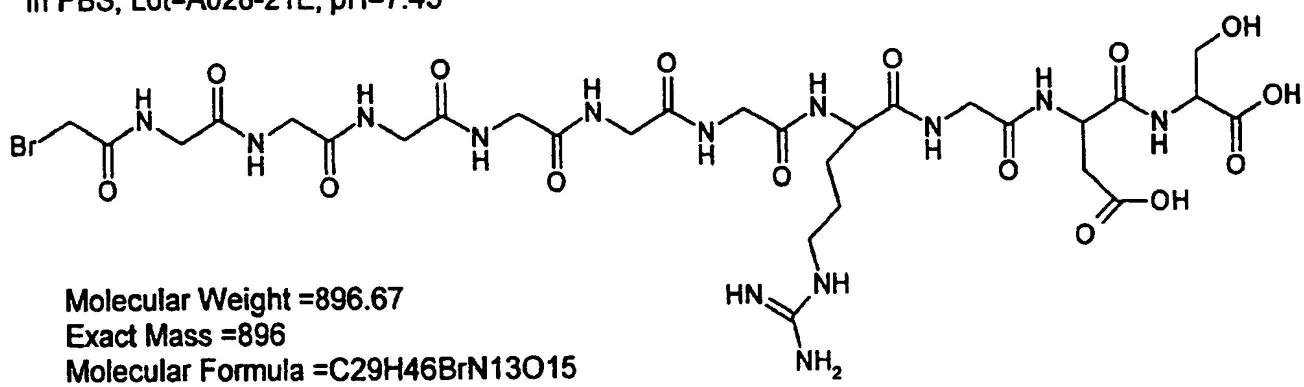
Molecular Weight =782.57
Exact Mass =782
Molecular Formula =C25H40BrN11O13

0.5 mL of Solution of 1 mMolar Ligand (lot A028-03)
in PBS, Lot=A028-21D; pH=7.45



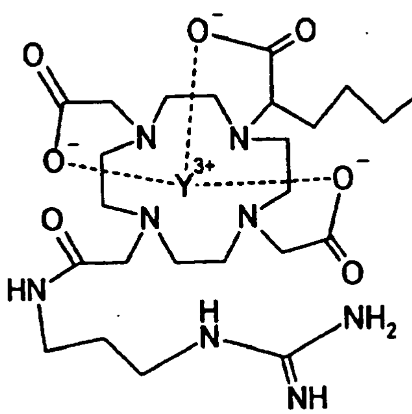
Molecular Weight =839.62
Exact Mass =839
Molecular Formula =C27H43BrN12O14

0.5 mL of Solution of 1 mMolar Ligand (lot A028-05)
in PBS, Lot=A028-21E; pH=7.45



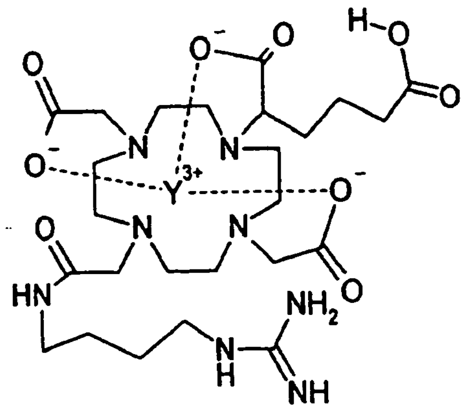
Molecular Weight =896.67
Exact Mass =896
Molecular Formula =C29H46BrN13O15

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16M



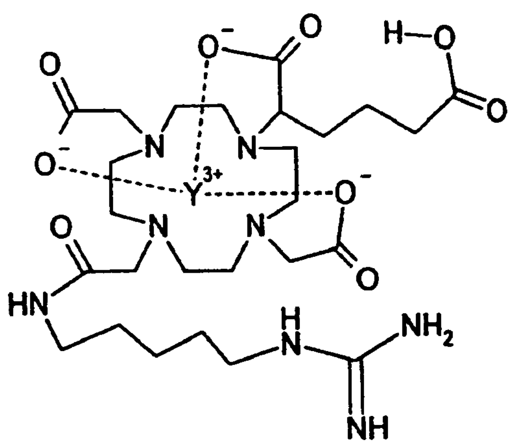
Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16N



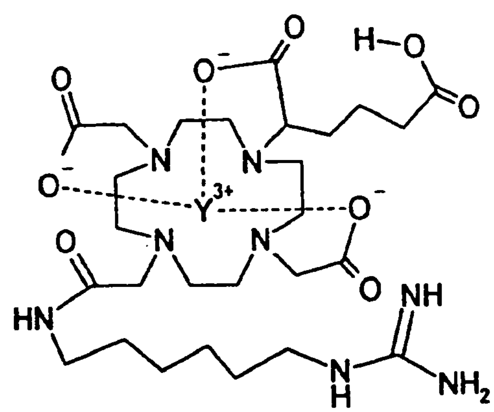
Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16O



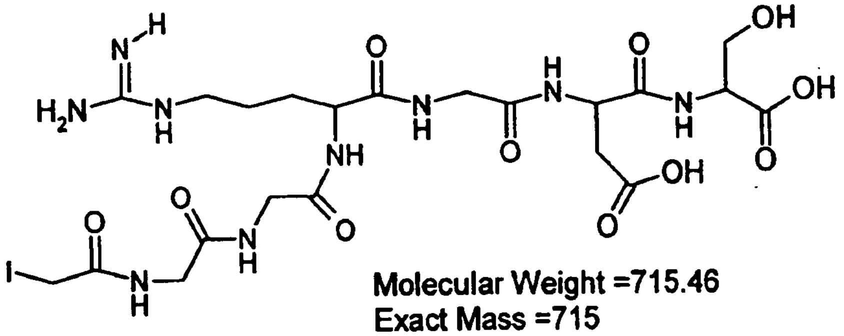
Molecular Weight =702.60
Exact Mass =702
Molecular Formula =C26H45N8O9Y

500 uL of 1 mMolar Complex in PBS
Lot Number= A024-16P



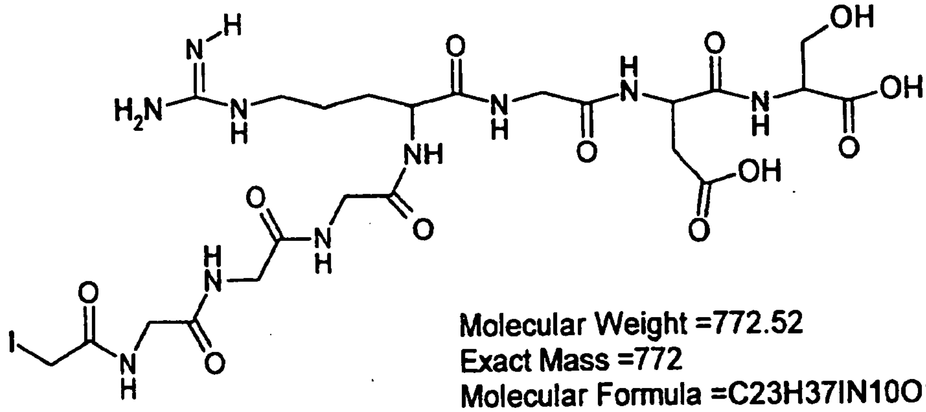
Molecular Weight =716.63
Exact Mass =716
Molecular Formula =C27H47N8O9Y

0.5 mL of Solution of 1 mMolar Ligand (lot A028-07)
in PBS, Lot=A028-22A; pH=7.41



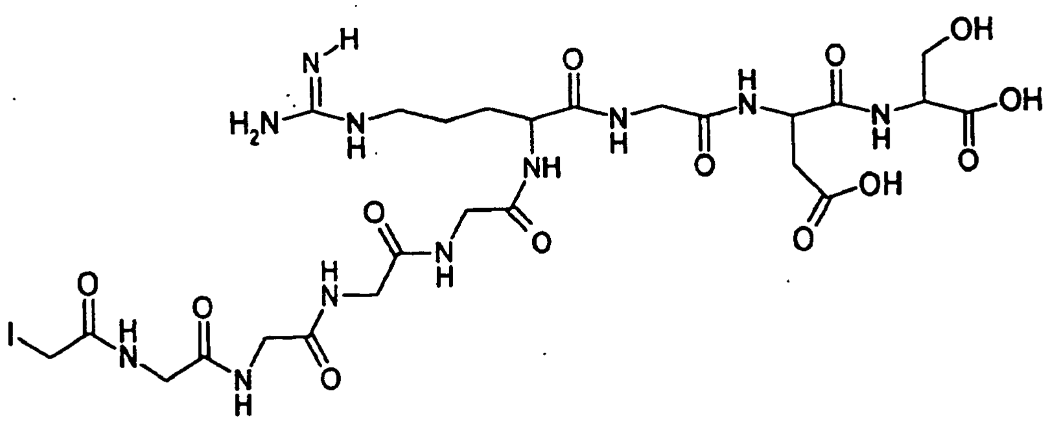
Molecular Weight =715.46
Exact Mass =715
Molecular Formula =C21H34IN9O11

0.5 mL of Solution of 1 mMolar Ligand (lot A028-09)
in PBS, Lot=A028-22B; pH=7.38



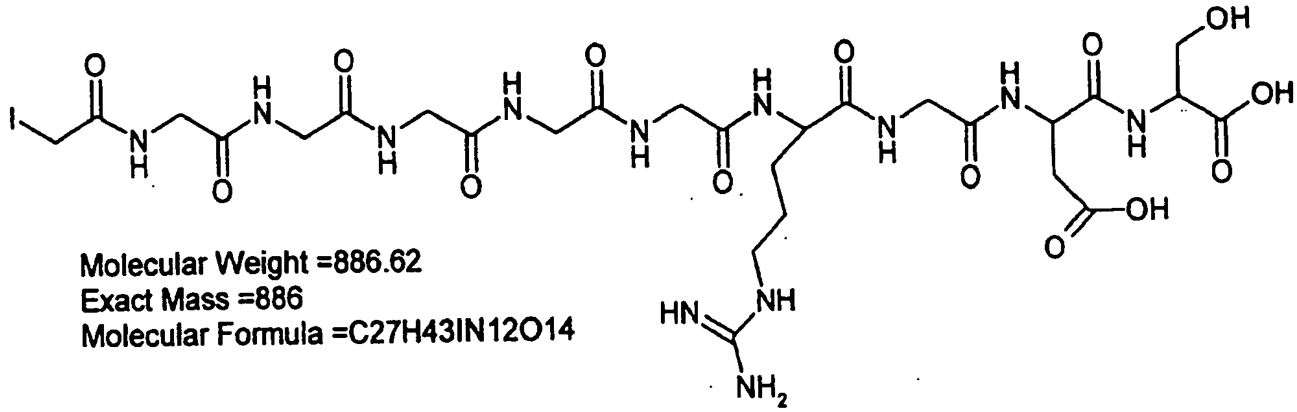
Molecular Weight =772.52
Exact Mass =772
Molecular Formula =C23H37IN10O12

0.5 mL of Solution of 1 mMolar Ligand (lot A028-11)
in PBS, Lot=A028-22C; pH=7.42



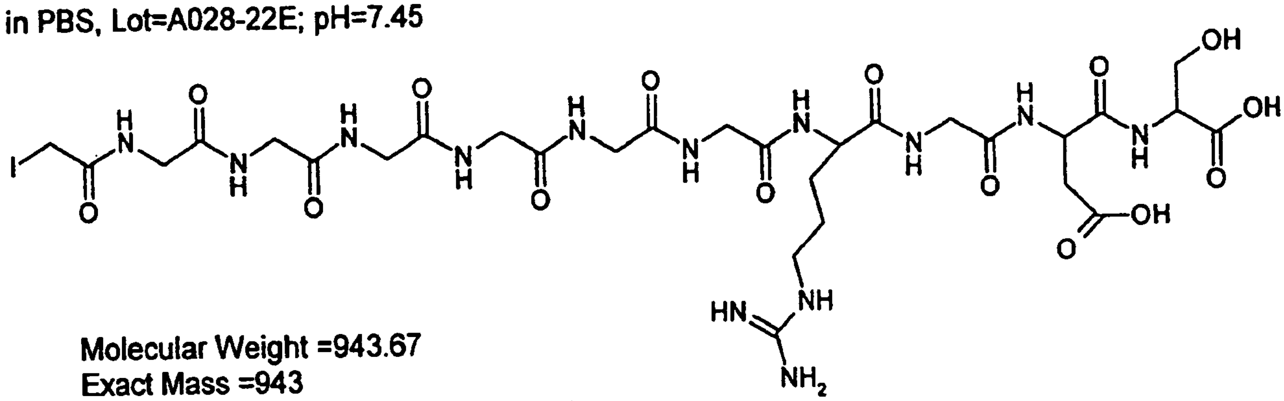
Molecular Weight =829.57
Exact Mass =829

0.75 mL of Solution of 1 mMolar Ligand (lot A028-13)
in PBS, Lot=A028-22D; pH=7.38



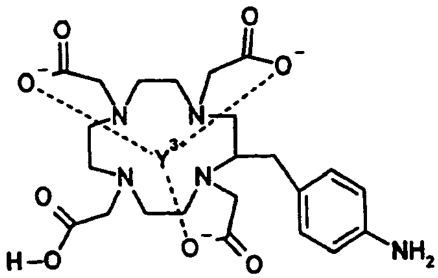
Molecular Weight =886.62
Exact Mass =886
Molecular Formula =C27H43IN12O14

0.5 mL of Solution of 1 mMolar Ligand (lot A028-15)
in PBS, Lot=A028-22E; pH=7.45



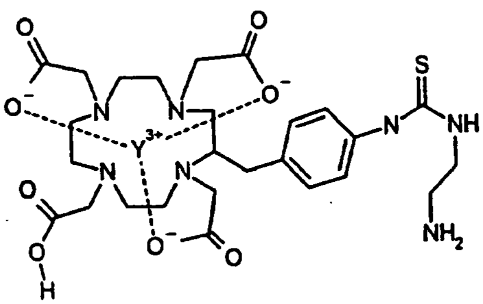
Molecular Weight =943.67
Exact Mass =943
Molecular Formula =C29H46IN13O15

Solution of 1 mMolar Complex in PBS = A007-26A
Ligand= lot pNH2–Benzyl-DOTA
Complex= A007-24



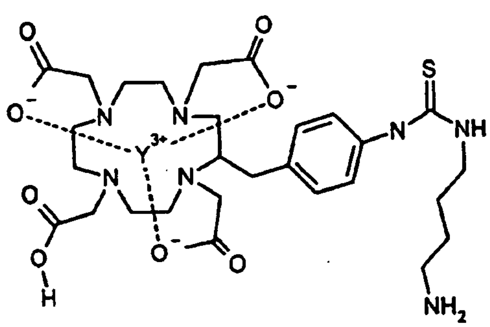
Molecular Weight =595.45
Exact Mass =595
Molecular Formula =C23H32N5O8Y

Solution of 1mMolar Complex in PBS= A007-40A
Ligand = lot A008-43
Complex= lot A007-37



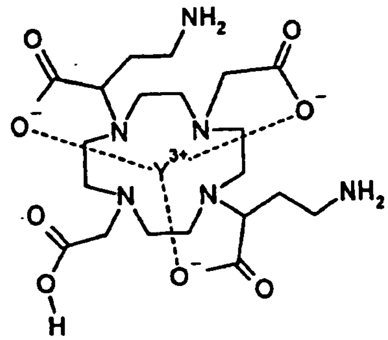
Molecular Weight =697.60
Exact Mass =697
Molecular Formula =C26H38N7O8SY

Solution of 1mMolar Complex in PBS= A007-35A
Ligand = lot A007-29
Complex= lot A007-35



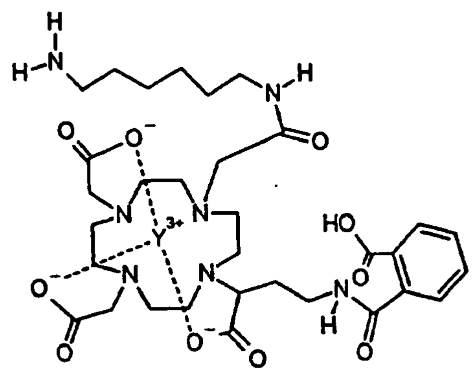
Molecular Weight =725.66
Exact Mass =725
Molecular Formula =C28H42N7O8SY

Solution of 1mMolar Complex in PBS= A007-58A
Ligand = lot A008-59
Complex= lot A007-51



Molecular Weight =576.44
Exact Mass =576
Molecular Formula =C20H35N6O8Y

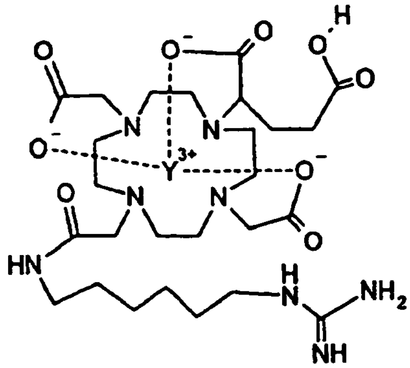
Solution of 1mMolar Complex in PBS= A007-36A
Ligand= lot A007-26
Complex = lot A007-27



Molecular Weight =779.69
Exact Mass =779
Molecular Formula =C32H48N7O10Y

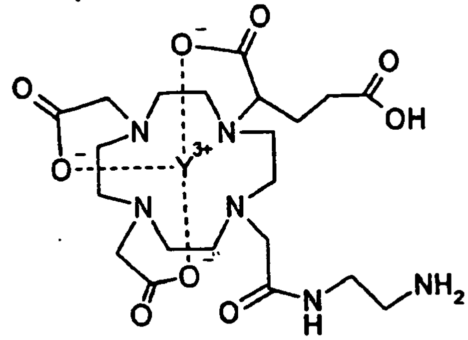
CONFIDENTIAL COMCHEM TECHNOLOGIES INC. [redacted]

0.189 mL of Solution of 1mMolar Complex in PBS=A019-42RS
Complex= Lot A013-97



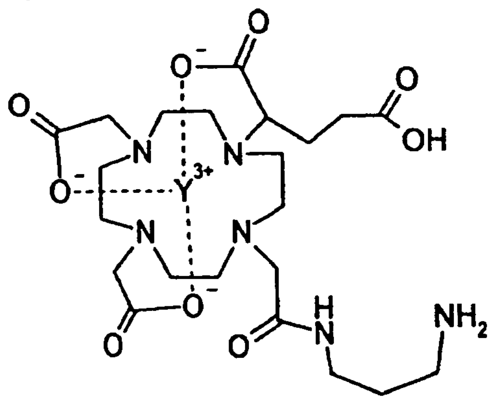
Molecular Weight =702.60
Exact Mass =702
Molecular Formula =C26H45N8O9Y

Solution of 1mMolar Complex in PBS=A019-38A
Ligand= Lot A017-21A
Complex= Lot A019-18



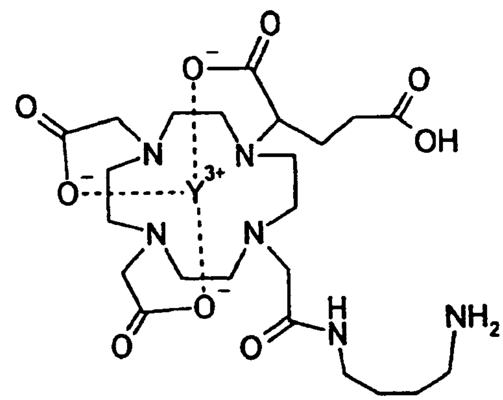
Molecular Weight =604.45
Exact Mass =604
Molecular Formula =C21H35N6O9Y

Solution of 1mMolar Complex in PBS=A019-38B
Ligand= Lot A017-21B
Complex= Lot A019-20



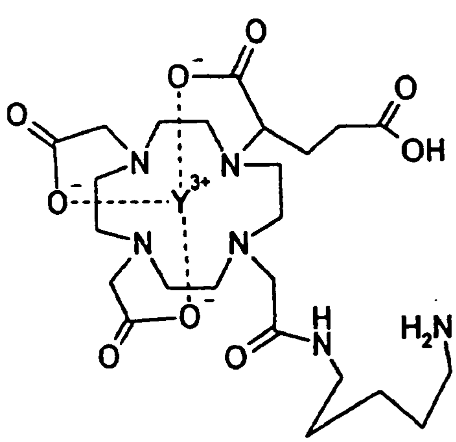
Molecular Weight =618.48
Exact Mass =618
Molecular Formula =C22H37N6O9Y

Solution of 1mMolar Complex in PBS=A019-38C
Ligand= Lot A017-21C
Complex= Lot A019-22



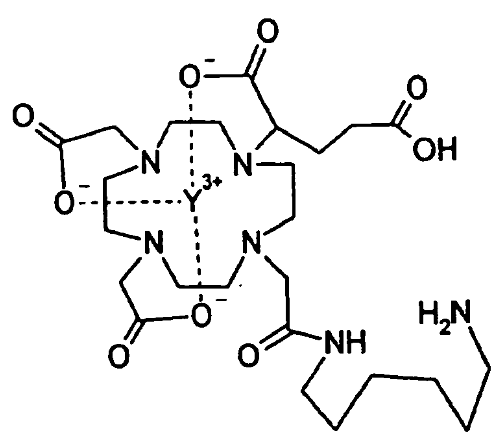
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS=A019-39A
Ligand= Lot A017-21D
Complex= Lot A019-24



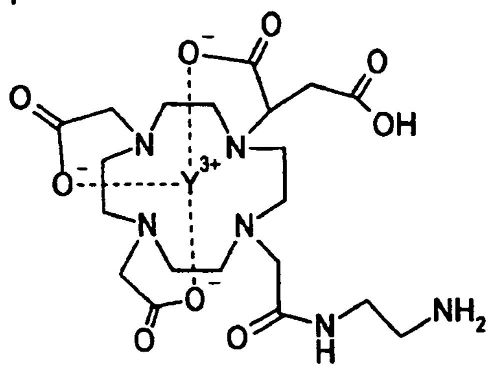
Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS=A019-39B
Ligand= Lot A017-21E
Complex= Lot A019-26



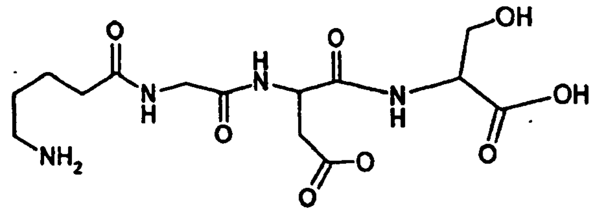
Molecular Weight =660.56
Exact Mass =660
Molecular Formula =C25H43N6O9Y

Solution of 1mMolar Complex in PBS=A019-39C
Ligand= Lot A017-25A
Complex= Lot A019-28



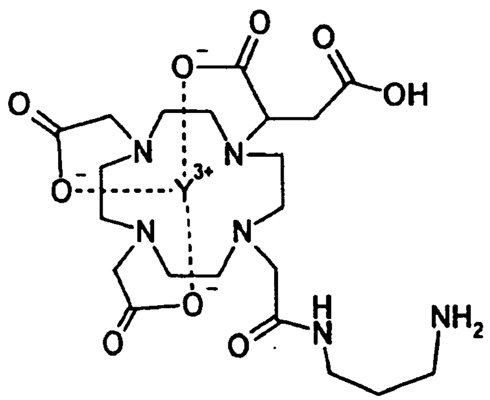
Molecular Weight =590.43
Exact Mass =590
Molecular Formula =C20H33N6O9Y

3.72 mL Solution of 1mMolar Complex in PBS=Lot A015-82PBS



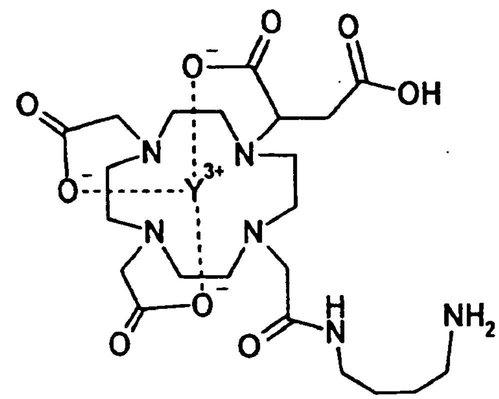
Molecular Weight =376.37
Exact Mass =376
Molecular Formula =C14H24N4O8

Solution of 1mMolar Complex in PBS=A019-39D
Ligand= Lot A017-25B
Complex= Lot A019-30



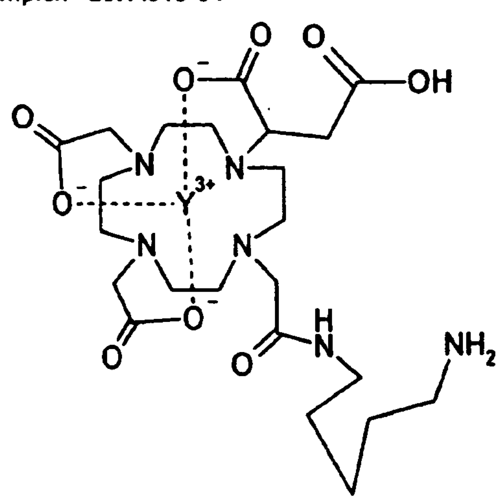
Molecular Weight =604.45
Exact Mass =604
Molecular Formula =C21H35N6O9Y

Solution of 1mMolar Complex in PBS=A019-44A
Ligand= Lot A017-25C
Complex= Lot A019-32



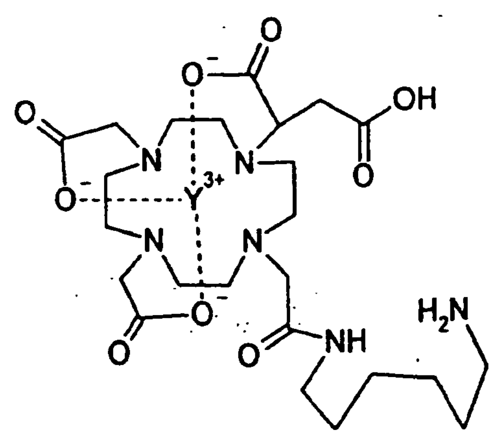
Molecular Weight =618.48
Exact Mass =618
Molecular Formula =C22H37N6O9Y

Solution of 1mMolar Complex in PBS=A019-44B
Ligand= Lot A017-25D
Complex= Lot A019-34



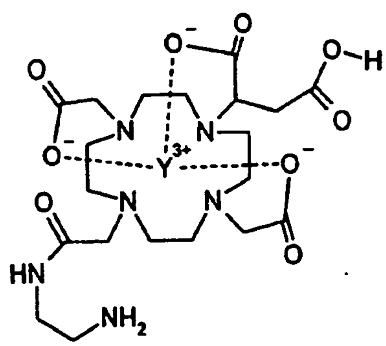
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS=A019-44C
Ligand= Lot A017-25E
Complex= Lot A019-36



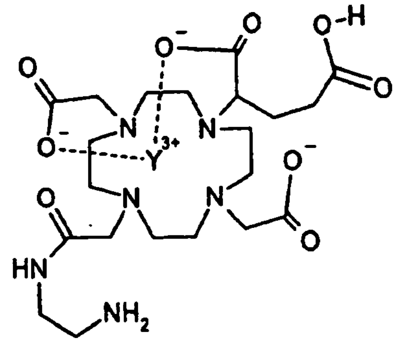
Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS=A012-93C
Ligand= Lot A011-97F
Complex= Lot A012-87



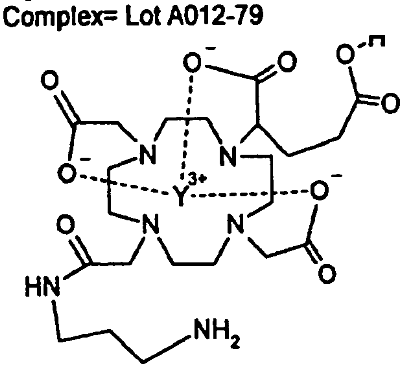
Molecular Weight =590.43
Exact Mass =590
Molecular Formula =C20H33N6O9Y

Solution of 1mMolar Complex in PBS=A012-92A
Ligand= Lot A011-97A
Compl x= Lot A012-77



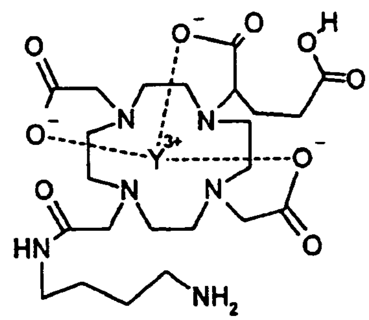
Molecular Weight =604.45
Exact Mass =604
Molecular Formula =C21H35N6O9Y

Solution of 1mMolar Complex in PBS=A012-92B
Ligand= Lot A011-97B
Complex= Lot A012-79



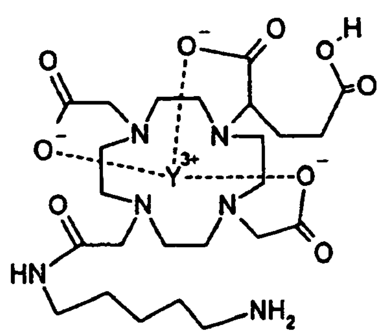
Molecular Weight =618.48
Exact Mass =618
Molecular Formula =C22H37N6O9Y

Solution of 1mMolar Complex in PBS= A012-92C
Ligand= Lot A011-97C
Complex= Lot A012-81



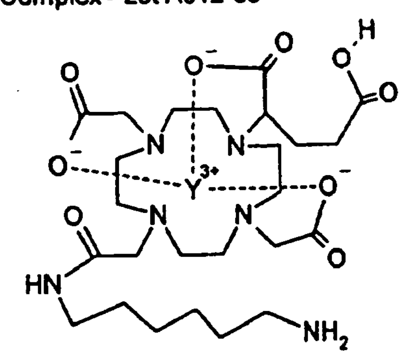
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS= A012-93A
Ligand= Lot A011-97D
Complex= Lot A012-83



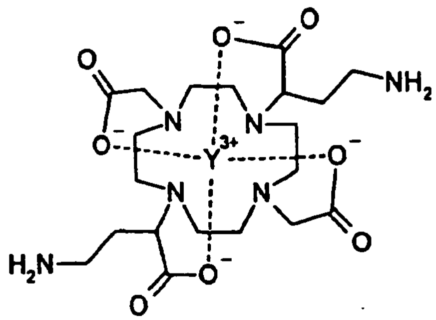
Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS= A012-93B
Ligand= Lot A011-97E
Complex= Lot A012-85



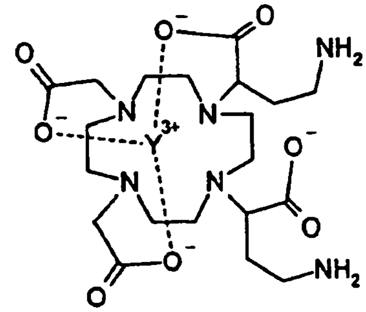
Molecular Weight =660.56
Exact Mass =660
Molecular Formula =C25H43N6O9Y

Solution of 1mMolar Complex in PBS=A016-13A
Ligand= Lot A011-35
Complex= Lot A016-2



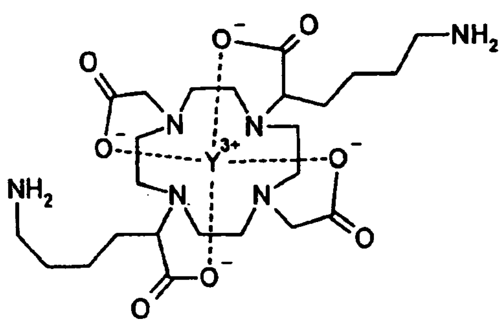
Molecular Weight =575.43
Exact Mass =575
Molecular Formula =C20H34N6O8Y

Solution of 1mMolar Complex in PBS=A016-13B
Ligand= Lot A013-17
Complex= Lot A016-4



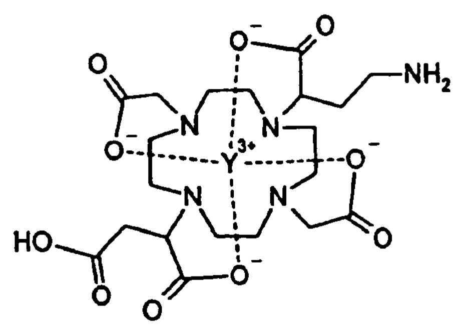
Molecular Weight =575.43
Exact Mass =575
Molecular Formula =C20H34N6O8Y

Solution of 1mMolar Complex in PBS=A016-14A
Ligand= Lot A013-19
Complex= Lot A016-6



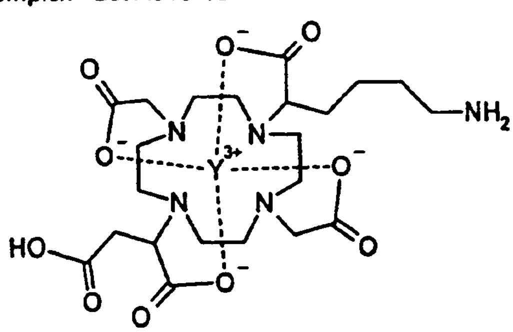
Molecular Weight =631.54
Exact Mass =631
Molecular Formula =C24H42N6O8Y

Solution of 1mMolar Complex in PBS= A016-14B
Ligand= Lot A013-25
Complex= Lot A016-8



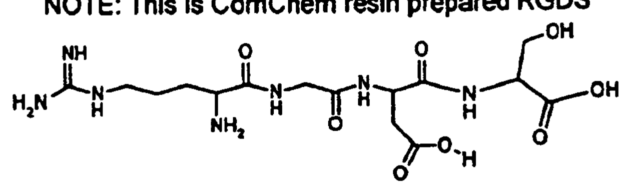
Molecular Weight =590.40
Exact Mass =590
Molecular Formula =C20H31N5O10Y

Solution of 1mMolar Complex in PBS= A016-14C
Ligand= Lot A013-27
Complex= Lot A016-10



Molecular Weight =618.46
Exact Mass =618
Molecular Formula =C22H35N5O10Y

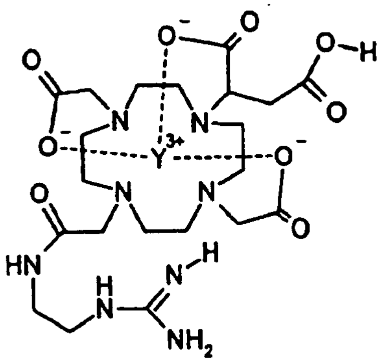
Solution of 1mMolar Complex in PBS= A016-14D
Ligand= Lot A015-2
NOTE: This is ComChem resin prepared RGDS



Molecular Weight =433.42
Exact Mass =433
Molecular Formula =C15H27N7O8

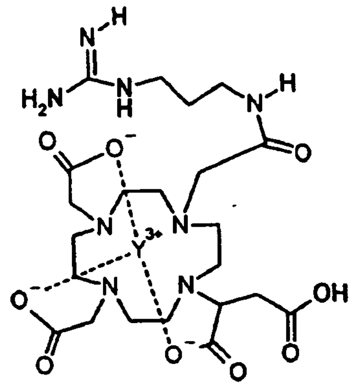
Compounds for Testing [redacted] Confidential Information

500 uL Solution of 1mMolar Complex in PBS= A017-50E



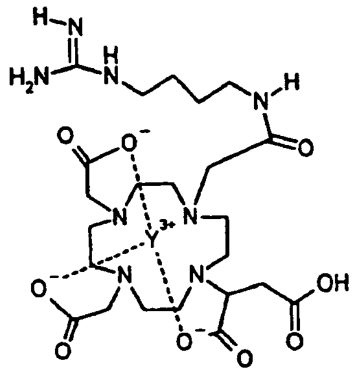
Molecular Weight =632.47
Exact Mass =632
Molecular Formula =C21H35N8O9Y

500 uL Solution of 1mMolar Complex in PBS= A017-50A



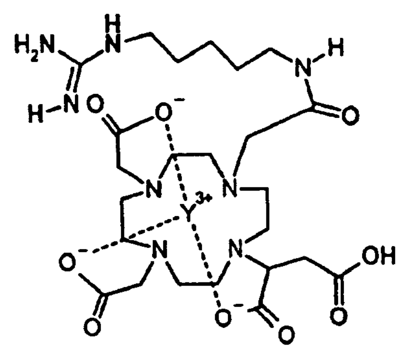
Molecular Weight =646.49
Exact Mass =646
Molecular Formula =C22H37N8O9Y

500 uL Solution of 1mMolar Complex in PBS= A017-50B



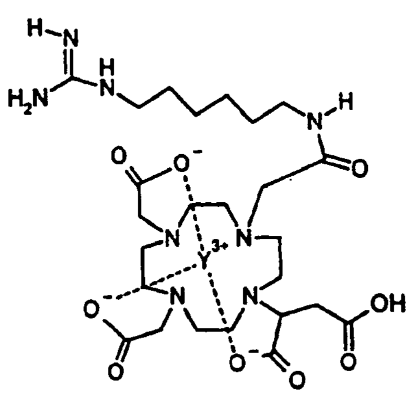
Molecular Weight =660.52
Exact Mass =660
Molecular Formula =C23H39N8O9Y

500 uL Solution of 1mMolar Complex in PBS= A017-50C



Molecular Weight =674.55
Exact Mass =674
Molecular Formula =C24H41N8O9Y

500 uL Solution of 1mMolar Complex in PBS= A017-50D

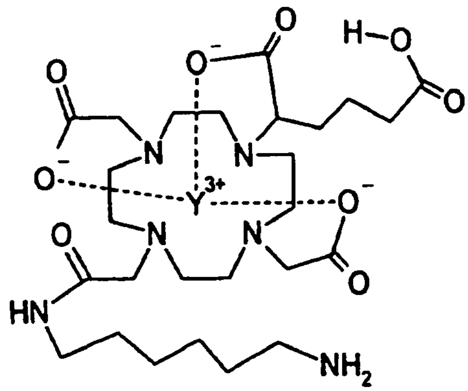


Molecular Weight =688.57
Exact Mass =688
Molecular Formula =C25H43N8O9Y

500 uLSolution of 1mMolar Complex in PBS= A017-50F
Control solution with Et3N and cyanamide

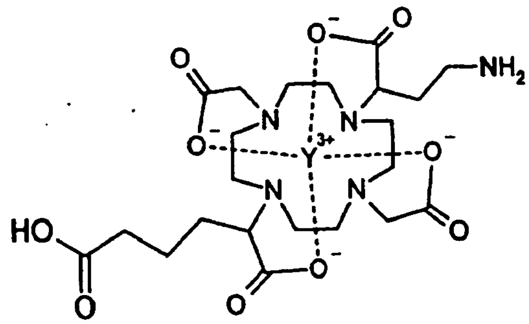
1.795 mL of 1 mMolar RGDS deriv in PBS= A017-48PBS

Solution of 1mMolar Complex in PBS=A016-62A
Ligand= Lot A013-67E
Complex= Lot A016-54



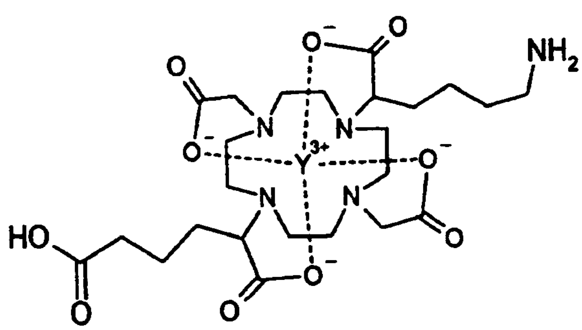
Molecular Weight =674.59
Exact Mass =674
Molecular Formula =C26H45N6O9Y

Solution of 1mMolar Complex in PBS=A016-62B
Ligand= Lot A013-77
Complex= Lot A016-56



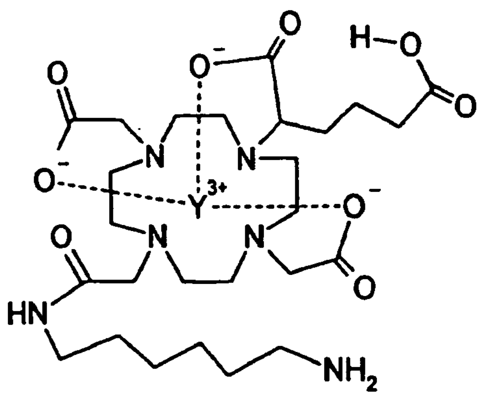
Molecular Weight =618.46
Exact Mass =618
Molecular Formula =C22H35N5O10Y

Solution of 1mMolar Complex in PBS=A016-62C
Ligand= Lot A013-79
Complex= Lot A016-58



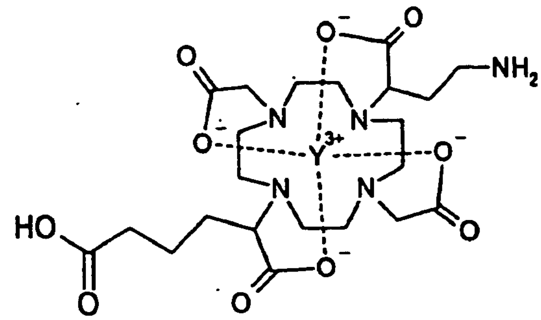
Molecular Weight =646.51
Exact Mass =646
Molecular Formula =C24H39N5O10Y

Solution of 1mMolar Complex in PBS=A016-62A
Ligand= Lot A013-67E
Complex= Lot A016-54



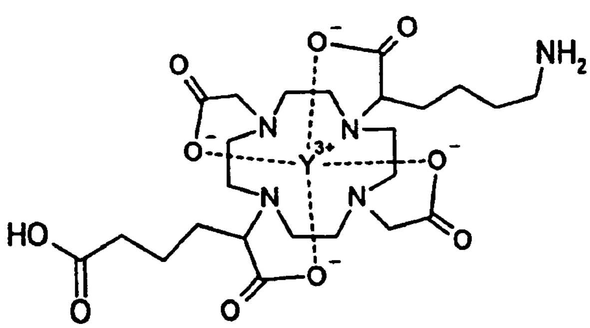
Molecular Weight =674.59
Exact Mass =674
Molecular Formula =C26H45N6O9Y

Solution of 1mMolar Complex in PBS=A016-62B
Ligand= Lot A013-77
Complex= Lot A016-56



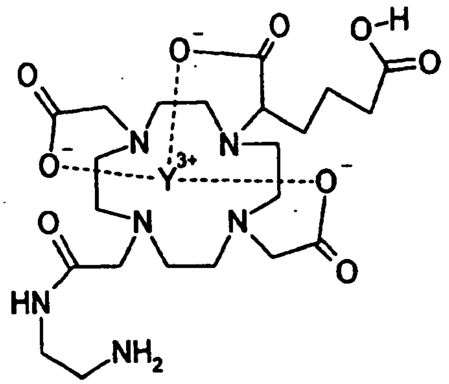
Molecular Weight =618.46
Exact Mass =618
Molecular Formula =C22H35N5O10Y

Solution of 1mMolar Complex in PBS=A016-62C
Ligand= Lot A013-79
Complex= Lot A016-58



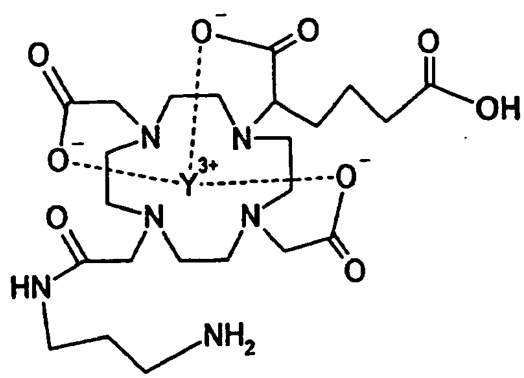
Molecular Weight =646.51
Exact Mass =646
Molecular Formula =C24H39N5O10Y

Solution of 1mMolar Complex in PBS=A016-61A
Ligand= Lot A013-67A
Complex= Lot A016-46



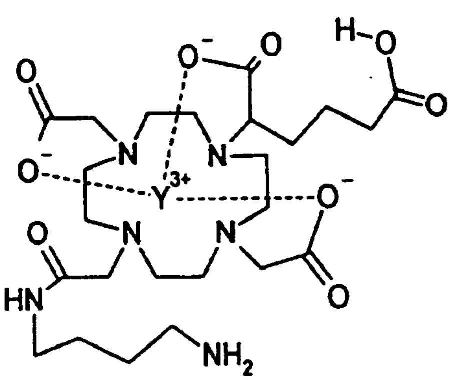
Molecular Weight =618.48
Exact Mass =618
Molecular Formula =C22H37N6O9Y

Solution of 1mMolar Complex in PBS=A016-61B
Ligand= Lot A013-67B
Complex= Lot A016-48



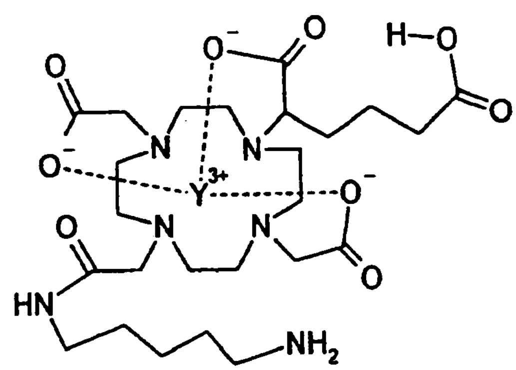
Molecular Weight =632.51
Exact Mass =632
Molecular Formula =C23H39N6O9Y

Solution of 1mMolar Complex in PBS=A016-61C
Ligand= Lot A013-67C
Complex= Lot A016-50



Molecular Weight =646.53
Exact Mass =646
Molecular Formula =C24H41N6O9Y

Solution of 1mMolar Complex in PBS=A016-61D
Ligand= Lot A013-67D
Complex= Lot A016-52



Molecular Weight =660.56
Exact Mass =660
Molecular Formula =C25H43N6O9Y

ComChem Technologies, Inc.
8496 Georgetown Road
Indianapolis, IN 46268

[redacted]

National Cancer Institute
Records Management Center
Executive Plaza South T42
6120 Executive BLVD
Rockville, MD 20852

Please find the original and two complete copies of our application for continuation/progress report for our grant entitled "Chelate Based Scaffolds (Chelabody) In Tumor Targeting". The grant number is R41CA92835.

Please let me know if there is any additional information needed to secure the second year of funding for this grant.

Thanks in advance,

Joseph R. Garlich

Joseph R. Garlich, Ph.D.
Chief Scientific Officer

Ph. 317-876-3075 Fax 317-872-1379 www.comchemtech.com

Form Approved Through 5/2004

OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number
ZCA1SRR B-E(M1)	5	R41	[redacted]

Total Project Period
From: [redacted] Through: [redacted]

Requested Budget Period:
From: [redacted] Through: [redacted]

1. TITLE OF PROJECT
Chelate Based Scaffolds (Chelabody) In Tumor Targeting

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
(Name and address, street, city, state, zip code)
Garlich, Joseph R.
ComChem Technologies Inc.
8496 Georgetown Road
Indianapolis, IN 46268

3. APPLICANT ORGANIZATION
(Name and address, street, city, state, zip code)
ComChem Technologies Inc.
8496 Georgetown Road
Indianapolis, IN 46268

2b. E-MAIL ADDRESS
garlich@comchemtech.com

4. ENTITY IDENTIFICATION NUMBER
1352100628A1

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

2d. MAJOR SUBDIVISION

5. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Executive Vice President
ComChem Technologies Inc.
8496 Georgetown Road
Indianapolis, IN 46268
E-MAIL: dreikorn@comchemtech.com

6. HUMAN SUBJECTS
☒ No ☐ Yes
6a. Research Exempt ☐ No ☐ Yes
6b. Human Subjects Assurance No.
If Exempt ("Yes" in 6a): Exemption No.
6c. NIH-Defined Phase III Clinical Trial ☐ No ☐ Yes
If Not Exempt ("No" in 6a): IRB approval date ☐ Full IRB or ☐ Expedited Review

7. VERTEBRATE ANIMALS
☒ No ☐ Yes
7a. If "Yes," IACUC approval Date
7b. Animal Welfare Assurance No.

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD
8a. DIRECT $225,111
8b. TOTAL $225,111

9. INVENTIONS AND PATENTS
☒ No ☐ Yes If "Yes," ☐ Previously Reported ☐ Not Previously Reported

10. PERFORMANCE SITE(S) *(Organizations and addresses)*
Department of Med. Chem & Mol. Pharmacology
Purdue University
1333 Pharmacy Building, Room 308
West Lafayette, IN 47907-1333

ComChem Technologies Inc.
8496 Georgetown Road
Indianapolis, IN 46268

11a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*
Joseph R. Garlich
TEL 317-876-3075
FAX 317-872-1379

11b. ADMINISTRATIVE OFFICIAL NAME *(Item 5)*
Barry A. Dreikorn
TEL 317-876-3075
FAX 317-872-1379

11c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 14)*
NAME Barry A. Dreikorn
TITLE Executive Vice President
TEL 317-876-3075 | FAX 317-872-1379
E-MAIL dreikorn@comchemtech.com

12. Corrections to Page 1 Face Page

13. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 2a. *(In ink. "Per" signature not acceptable.)*
Joseph R. Garlich

DATE [redacted]

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11c. *(In ink. "Per" signature not acceptable.)*
Barry Dreikorn

DATE [redacted]

Principal Investigator/Program Director (Last, first, middle): Garlich, Joseph R.

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	[redacted]	[redacted]	[redacted]

PERSONNEL (Applicant organization only)				DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Joseph Garlich, PhD	Principal Investigator	12	40.0	41,661	2,796	44,457
Mary Patterson,PhD	Res. Associate	12*	50.0	23,175	2,820	25,995
Bob Suhr, M.S.	Res. Associate	12*	100.0	37,080	0	37,080
SUBTOTALS →				101,961	5,616	107,532

CONSULTANT COSTS Dr. Donald Durden, M.D., Ph.D., Indiana Univ. School of Medicine (3 daysX$1000/day)	3,000
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* $13,000 chemicals and disposable chemistry supplies $3,480 glass ware and disposable plasticware	16,480
TRAVEL	
PATIENT CARE COSTS — INPATIENT	
PATIENT CARE COSTS — OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	**$127,012**
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	65,399
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	32,700
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD *(Item 9a, Face Page)*	**$225,111**

Principal Investigator/Program Director (Last, first, middle): Garlich, Joseph R.

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	[redacted]	[redacted]	[redacted]

PERSONNEL (Applicant organization only)		TYPE APPT. (months)	% EFFORT ON PROJ.	DOLLAR AMOUNT REQUESTED (omit cents)		
NAME [Contractual Budget; Purdue]	ROLE ON PROJECT			SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Mark Green, Ph.D.	Principal Investigator	12	10.0	11,263	3,672	14,935
Alfred C. Dumaual	post-doc	12	100.0	30,150	10,914	41,064
Carla Mathias	Proj. Coord.	12	5.0	3,086	1,203	4,289
SUBTOTALS →				44,499	15,789	60,288

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Assay costs, disposables, solvents, counting supplies	5,111
TRAVEL	
PATIENT CARE COSTS — INPATIENT	
PATIENT CARE COSTS — OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	**$65,399**
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	32,700
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD *(Item 9a, Face Page)*	**$98,099**

Principal Investigator/Program Director (Last, first, middle): Garlich, Joseph R.

BUDGET JUSTIFICATION	GRANT NUMBER [redacted]

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

ComChem Technologies Inc. : The second year budget is completely in line with the budget previously recommended.

Contractual Budget (Purdue University): The second year budget for the contractual organization is completely in line with the budget previously recommended.

*The supporting scientists for ComChem's part of the budget marked by an asterisk are less than full-time employees. This allows ComChem to take advantage of the varied expertise found in these very experienced people (for example synthesis skills and analytical/complexation skills). ComChem would not be able to afford hiring both of these scientists fulltime so by employing them each half-time we can afford both on our budget and bring all of their experience to bear on this project.

CURRENT BUDGET PERIOD	FROM [redacted]	THROUGH [redacted]

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

The unobligated budget will not exceed 25% of the current year's total budget.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sampl format for each person. DO NOT EXCEED FOUR PAGES.

NAME	POSITION TITLE
Alfred C. Dumaual	Postdoctoral Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Notre Dame, IN	B.S.	1990	Biology
Purdue University, IN	M.S.	1993	Biology
Indiana University School of Medicine, IN	Ph.D.	2000	Medical Biophysics

A. Positions and Honors:

Postdoctoral Research Associate, Purdue University, Department of Industrial and Physical Pharmacy 2001-Present
Development and screening of RGD peptide mimetics for the detection and treatment of brain tumors.

Postdoctoral Research Associate, University of Virginia, Department of Pharmacology 1999 – 2001
Analysis of lipid raft formation during apoptosis and cell signaling.
Characterization of the effects of lipid rafts on Annexin V binding

Teaching Assistantship, Indiana University-Purdue University at Indianapolis (IUPUI)

Laboratory in Human Biology, Biology Department	1997 – 1999
Human Biology, Biology Department	1998 – 1999
Introductory Biology Laboratory, Biology Department	1991 – 1997
Introductory Biology, Biology Department	1992 – 1997

HONORS:
IUPUI Travel Fellowship, March 1998
Phi Beta Psi, Fall 1996
Teaching In Excellence Award (TERA), Indiana University-Purdue University at Indianapolis, Indianapolis, IN. April 1997
Outstanding Graduate Teaching Assistant, Biology Department, Purdue School of Science at Indianapolis, Indianapolis, IN. April 1992

B. Publications:

1. Shaikh, S.R., Dumaual A.C., Jenski L.J. and Stillwell W. Lipid phase separation in phospholipid bilayers and monolayers modeling the plasma membrane. *Biochimica Biophysica Acta* 1512(2):317-28 (2001).
2. Dumaual, A.C., Jenski, L.J. and Stillwell, W. Lateral phase separation in docosahexaenoic acid-enriched PC monolayers. *Biochimica Biophysica Acta* 1463:395-406 (2000).
3. Stillwell, W., Jenski, L.J., Zerouga, M. and Dumaual, A.C. Detection of lipid domains in docosahexaenoic acid-rich bilayers by acyl chain-specific FRET probes. *Chemistry and Physics of Lipids* 104(2):113-132 (2000).

4. Schoefield, M., Jenski, L.J., Dumaual, A.C. and Stillwell, W. Cholesterol versus cholesterol sulfate: Effects on properties of phospholipid bilayers containing docosahexaenoic acid. *Chemistry and Physics of Lipids* 95:23-36 (1998).
5. Stillwell, W., Dallman, T., Dumaual, A.C., Crump, F.T. and Jenski, L.J. Cholesterol vs. α-tocopherol: Effect on properties of bilayers made from heteroacid phosphotidylcholines. *Biochemistry* 35:13353-13362 (1996).
6. Stillwell, W., Ehringer, W.D., Dumaual, A.C. and Wassall, S.R. Cholesterol condensation of α-linolenic and γ-linolenic monolayers and bilayers. *Biochimica Biophysica Acta* 1214:131-136 (1994).
7. Stillwell, W., Wassall, S.R., Dumaual, A.C., Ehringer, W.D., Browning, C.W. and Jenski, L.J. Use of merocyanine 540 (MC540) in quantifying lipid domains and packing in phospholipid vesicles and tumor cells. *Biochimica Biophysica Acta* 1146:136-144 (1993).

C. Research Support. No current independent support.

Grant Progress Report: OTHER SUPPORT
Grant Application Number :**1R41CA92835-02**
Grant Application Title: **Chelate Based Scaffolds (Chelabody) In Tumor Targeting**

GARLICH, JOSEPH R.
ACTIVE SUPPORT
1R43CA96259-01 [redacted] 10%
NIH/NCI SBIR Phase I $160,600
"Targeted Delivery of Protectant p53 Inhibitors"

The aim of this project is to prepare prodrugs of p53 inhibitors that target the bone marrow spaces in an effort to protect the bone marrow from chemotherapy and radiation therapy.

PENDING SUPPORT
1R43CA096080-01A1 1[redacted] 5%
NIH/NCI SBIR Phase I $173,295
"Anticancer Conjugates of PI3 Kinase Inhibitors"

The aim of this project is to prepare prodrugs of PI3 kinase inhibitors in an effort to target them to the tissues where they are needed to sensitize tumor cells toward chemotherapy and radiation therapy.

OVERLAP
None of the pending or active grant support specific aims overlap with the application under consideration.

GREEN, MARK A.
ACTIVE SUPPORT
R01-CA70845 [redacted] 27%
NIH/NCI $240,000
"Radiopharmaceuticals Targeted to Tumor Folate Receptors"

The major goals of theis project are the deisgn, synthesis, and evaluation of folate-chelate conjugates as ehicles for tumor-selective radionuclide delivery (targeting a tumor cell membrane-associated folate eceptor).

DE-FG01-01NE23050 [redacted] 5%
US Department of Energy $93,600
"Advanced Nuclear Medicine Initiative: Nuclear Pharmacy Educational Program"

This project supports development of laboratory and clinical training opportunities for students and harmacists interested in practicing nuclear pharmacy.

Grant Application Number : **1R41CA92835-02**
Other Support (Continued)

GREEN, MARK A. (continued)

PENDING SUPPORT
R01CA92403 [redacted] 20%
NIH/NCI $200,000
"PET Radiotracers to Evaluate Tumor Multidrug Resistance"

This project focuses on the synthesis and evaluation of radiolabeled metal chelates as PET radiopharmaceuticals to image MDR1 Pgp transport function.

OVERLAP
There is no overlap of the above grants specific aims with those of the current proposal under consideration.

PROGRESS REPORT SUMMARY	GRANT NUMBER CA92835-02	
	PERIOD COVERED BY THIS REPORT	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Joseph R. Garlich	FROM [redacted]	THROUGH [redacted]
APPLICANT ORGANIZATION ComChem Technologies Inc.		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Chelate Based Scaffolds (Chelabody) in Tumor Targeting		

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects ☐ No Change Since Previous Submission ☐ Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals ☐ No Change Since Previous Submission ☐ Change

A. Specific Aims. Due to the Study Section's deletion of the originally planned animal studies the revised specific aims for the two year period of this grant are:

1) Develop and communicate new solid-phase methodology for macrocyclic chelating agents.
2) Prepare avB3 integrin antagonists based around conformationally restricted chelating agent-metal ion complexes.
3) Design, construct, and test multivalent avB3 integrin receptor binding molecules.

We have made great progress in achieving #1 and #2. The third aim awaits additional progress in aim#2.

B. Studies and Results. We have chosen the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, 1) scaffold upon which to attach our molecular recognition units because of its known structural rigidity in solution coupled with its known *in vivo* stability with radioactive metal ions. The known required molecular recognition units for binding with the avB3 integrin receptor are an acidic group (such as carboxylic acid) and a basic group (such as an amine or guanidine) separated in space by 10 to 20 angstroms. The challenge is to synthesize DOTA-based chelating agents possessing acidic and basic pendant molecular recognition units with suitable orientation for avB3 binding. We have made tremendous progress in the synthesis of such chelating agents, have developed the synthetic protocols for library production, prepared a few final examples along the way, worked out complexation procedures with yttrium, and implemented and validated a biological avB3 binding test method for such complexes as summarized below:

Modeling Studies: We have performed extensive molecular modeling studies in support of our aims. It should be noted that the crystal structure of the extracellular segment of the avB3 receptor with an arginine-glycine-aspartic (RGD) amino acid containing ligand was published in the April 5th (2002) issue of Science. We have been able to refine and confirm our modeling hypotheses using the crystal structure of the basic group of arginine (of the bound RGD ligand) relative to the acidic aspartic acid group with the following conclusions: 1) Different metal ion size in the metal-DOTA complex do not significantly effect the spatial dispositions of the acetate arm substituents (i.e. changing the metal from Y+3 to Ho+3 would allow us to us a different therapeutic radioisotope with the same RGD mimetic); 2) The substitution pattern of 1,4 vs 1,7 on the tetraazamacrocyclic ring using substituted acetate arms both give plausible candidates for RGD mimetics; 3) The different stereoisomers due to the chirality introduced by a substituent on the chelating acetate arm points the substituent into different space but depending on how one orients the complex into the avB3 receptor site all possible stereoisomers can be positioned to mimic RGD; 4) Conversion of one of the chelating arms to an amide instead of carboxyl leads to additional novel compositions that we have shown can be potential RDG mimetics;

Synthetic Progress: We have made good synthetic methodology progress and have created the first examples of macrocyclic chelating agents containing a pendant acid and a pendent basic group as possible RGD mimetics.

We have completed the synthetic studies on the original proposed route to macrocyclic RGD chelator mimetics. Unfortunately this route suffered from premature cleavage from the solid phase resin coupled with extremely slow reaction rates. Additional work in traditional solution phase approach also indicated this route would be problematic even in solution phase. However, having performed these studies we were in a position to realize, based on the reactivity profiles we saw on solid support and in solution, just what chemistry would work to allow access to macrocyclic chelating agents containing a pendant acid and a pendent basic group as possible RGD mimetics. W have successfully pioneered two routes to such macrocyclic constructs. These routes are outlined in Figure 2 and 5.

Figure 2 shows the solid phase approach that we have developed. This route starts with Wang solid phase resin (2) and utilizes attachment of a symmetrical diacid to the resin (representing the first variable input). The diacid is then coupled with key bis-amine intermediate 4 via one of the amine groups to give 5 and then the other amine group of 5 can be left as-is or reacted further with a protected aminoacid (6) to extend out the chain length to give 7. This construct is then cleaved from the resin by exposure to trifluoroacetic acid which also deprotects the various amine and

carboxy protecting groups to yield the macrocylic chelator containing RGD mimetic groups (8). The scale we are working in is such that we end up with 2 to 10 milligrams of final chelator 8 suitable for preparing the metal complex for biological evaluation. We have prepared the novel key intermediate 4 in our lab in multi-hundred milligram quantities. We have prepared the 1,7-substituted tetraazamacrocycle precursor to 4 (14) in multigram quantities by the rout shown in Figure 4 starting with commercially available cyclen (15). This intermediate (4) represents a variable in chain length and orientation (i.e. 1,7 vs 1,4 substitution on the macrocycle ring). So far we hav successfully prepared a series of four bromo-compounds (13) in multigram quantities via the method shown in Figure 3 starting with amino acids, protecting with a phthalimido group, conversion to the acid chloride, bromination, and finally quenching in methanol. Reaction of excess bromo-compounds 13 with the disubstituted cyclen 18 gives good yields of the tetra-substituted cyclen 14. Complete removal of all the protecting groups was effected in 6N HCl with heating for extended times to give the key bis-amine 4 which can then be attached to resin 3. Once bound to resin the free amine group of 5 can be modified by chain extension using coupling of amine-protected amino acids (6, of which we have prepared five in multigram quantity) or the pendant amino group can be converted to a guanidine group (which we are still working on using various guanidinylating agents). Finally, we have demonstrated cleavage of such compounds (7) from the resin to give useful quantities of compounds represented by 8. We have been able to complex such resin cleaved product directly with yttrium metal ions. We are now cranking out the target compounds using these solid phase methods. The library size for this focused collection will be about 960 compounds using 12 diacids on resin (3), 4 bromo-compounds (13), 10 amino-acids (6), and the two positional isomers of the macrocyclic ring (1,4-appended versus 1,7-appended).

It should be noted that to make half of this library we also need access to the 1,4-substituted version of 1,7-substituted compound 18. We have found a novel chemical route to multigram quantities of this compound (18) and have proven that it is the desired 1,4-isomer. We will be submitting this route in the second year for publication.

Because the modeling studies support the rationale of preparing compounds such as 25 as RGD mimetics we have worked out another synthesis using solid phase resin shown in Figure 5. We have successfully completed the synthesis of one example of 25 just recently. We are now starting to make a library of about 80 compounds of this structure type(from 10 diamines, two positional isomers (1,4 and 1,7-substituted macrocycles), and four bromo-compounds).

Complexation studies: We have successfully worked out aqueous complexation chemisty procedures for the RGD mimetic chelating agents with trivalent nonradioactive yttrium.

Biological Assay Studies: A biological whole cell adhesion inhibiton assay has been developed to evaluate the synthesized macrocyclic RGD mimetics. This assay uses endothelial cells known to express avB3 receptors on their surface and also known to require vitronectin binding at the avb3 receptor to initiate adhesion processes. Vitronectin is coated on microtiter plates and exposed to cell suspensions. Thus, as our target molecules compete with vitronectin for binding at the avB3 they will interfere with the adhesion process and we can quantify/rank their ability to do so. The amount of avB3 mediated adhesion is determined by cell staining with subsequent quantitation by uv absorbtion proportional to the amount of stain present. We have optimized this test with regard to vitronectin quantities, cell numbers, volumes, times, and the cell staining process. We have validated this test with known avB3 antagonists (positive control) and known inactive analogs (negative controls). We have obtained IC_{50} values for known avB3 antagonists that are comparable to those reported in the literature. Our adhesion inhibition assay is very similar to one published in last month in the April issue of Bioconjugate Chemistry verifying that using whole cells expressing th avB3 receptor is a bioassay better linked to real world results versus the *in vitro* isolated receptor assays reported previously in the literature. We have run the first set of 12 target compounds (yttrium-complexes and free chelating agents) through our bioassay with no significant bioactivity so far. However, these first 12 compounds represent the fruits of the synthetic methodology work and not the best target compounds that we will be making as we prepare the libraries of compounds.

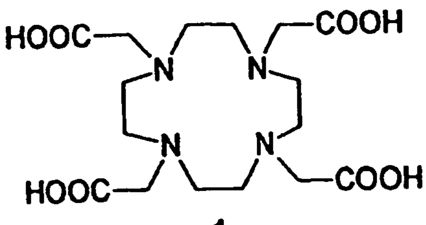
C. Significance. Our synthesis methodology revolving around macrocyclic complexes displaying various molecular recognition groups is of high value and utility in nuclear medicine for both diagnostic (magnetic resonance imaging agents including imaging molecular processes and gamma imaging) and therapeutic purposes (radioimmunotherapy, radiopharmaceuticals employing transition and lanthanide metal ions). Our final active avB3 binding agents will be the first example of the chelating agent serving as the scaffold for biomolecular recognition of metal complexes.

D. Plans. We will execute on the library production schemes described above to deliver target compounds, complex such compounds with yttrium and assay such complexes in our cell adhesion assay. We will also finish work on a bioassay using the same cell line that determines cell binding of our target molecules with the cell surface avB3 receptors. This cell binding assay will utilize radioactive metal ligand complexes and the challenge is how to do this with the large number of library compounds. The multivalent binding specific aim (#3) will be started as soon as a suitably bioactive library member is identified. Our plethora of chemistry that we have worked out so far in our macrocycle synthesis will enable us to prepare such constructs.

E. Publications. There have been no publications. There will be several submissions by the end of the second year as our focus in the first period has been to push through to get methodology to prepare target molecules.

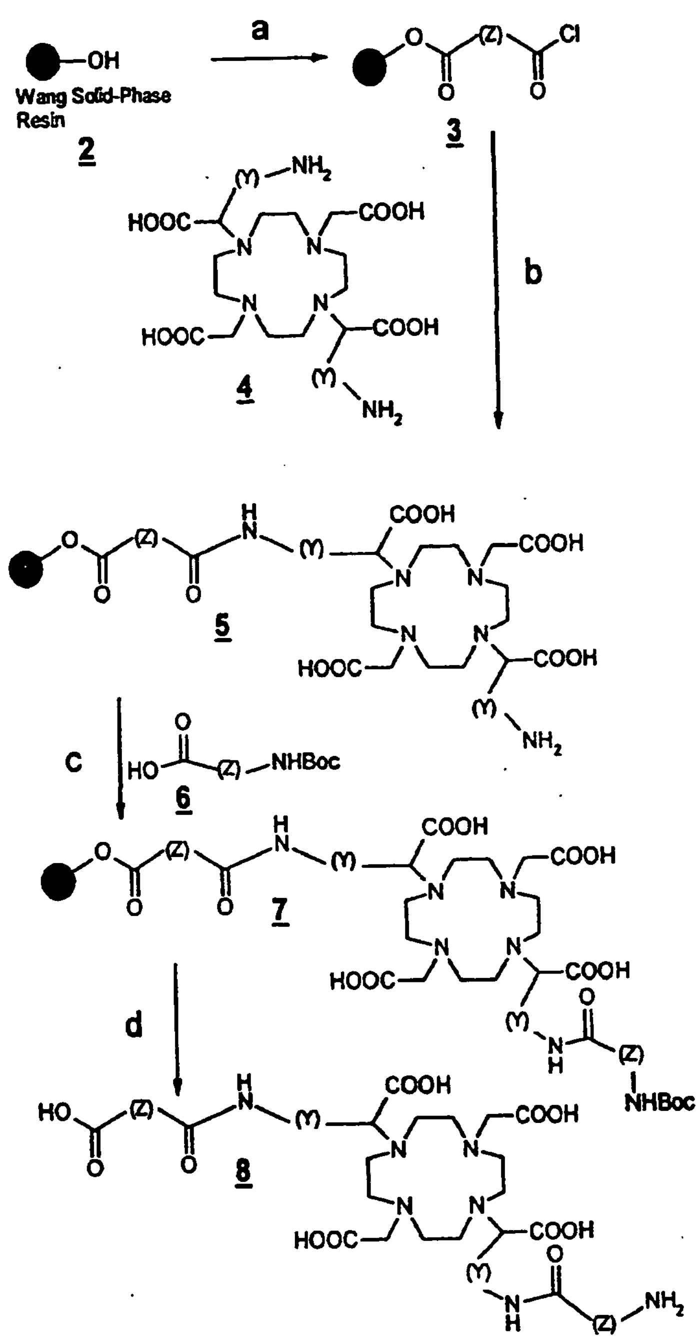
F. Project Generated Resources. Not applicabl

Figure 1: DOTA



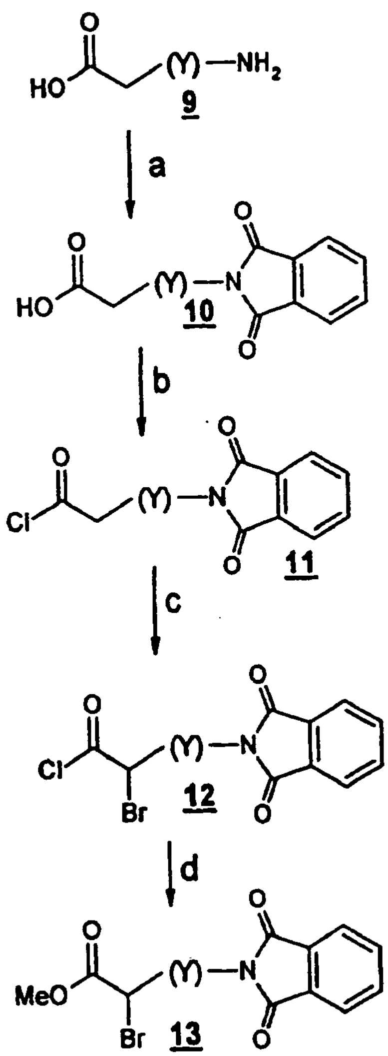
1
DOTA

Figure 2: Solid Phase Synthesis of Macrocyclic Chelator RGD Mimetics



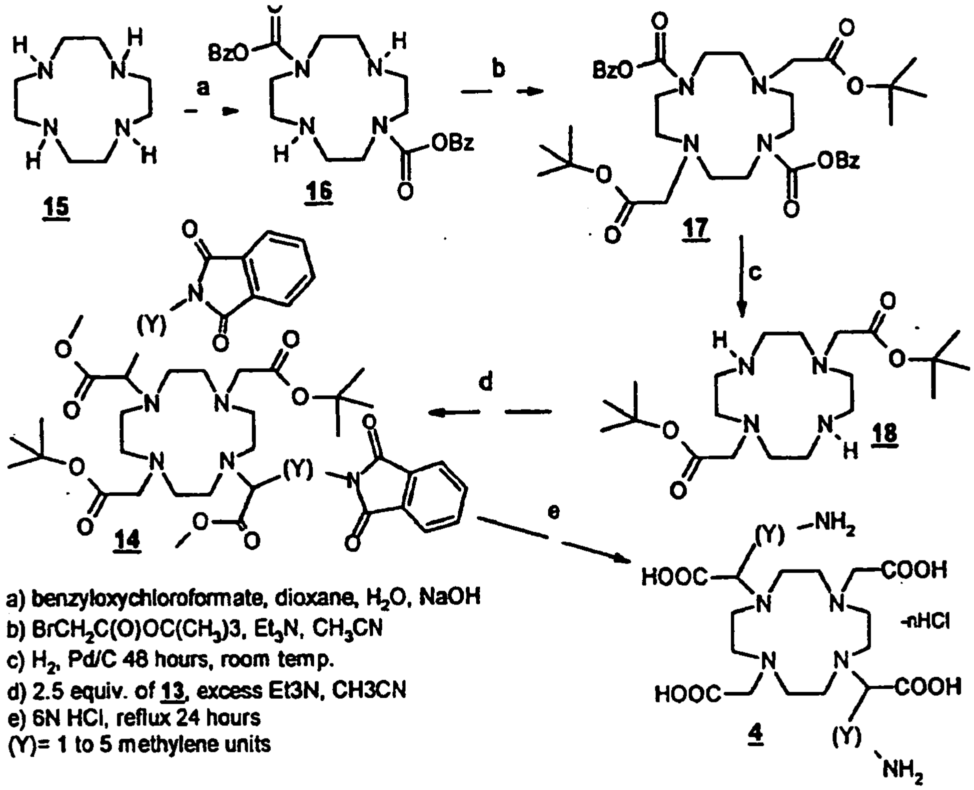
a) symmetrical acid chloride, pyridine, CH_2Cl_2;
b) DMF, Et_3N;
c) carbodiimide coupling or acid chloride;
d) Trifluoroacetic acid/CH_2Cl_2 50/50
(Y)= 1 to 5 methylen units
(Z)= variabl spacer groups

Figure 3: Synthesis of Chain Extenders



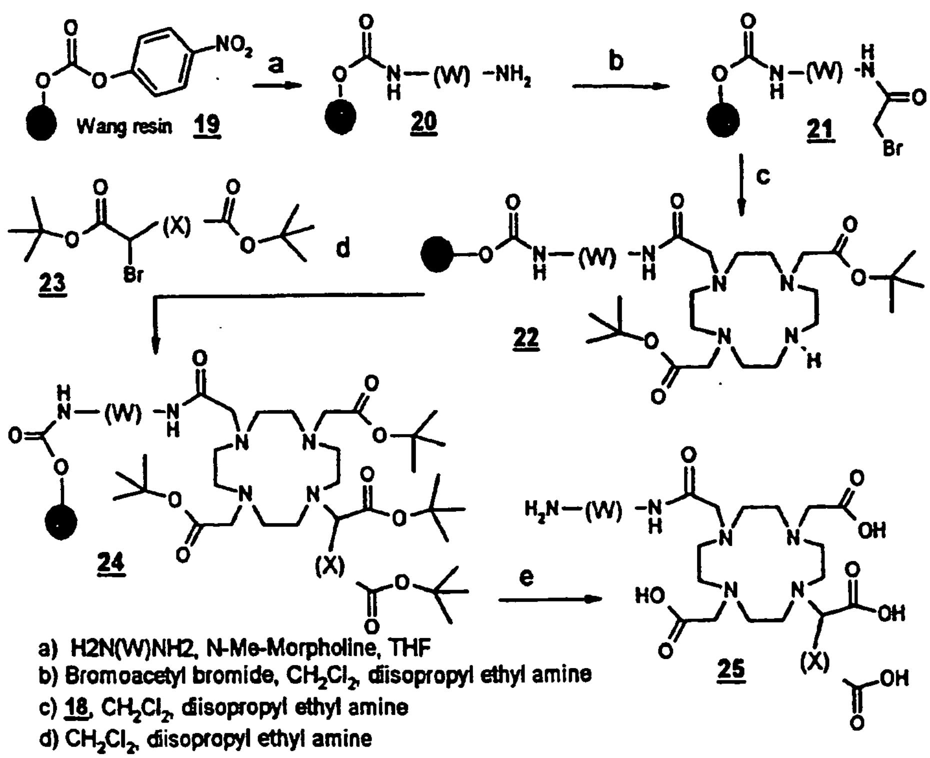
a) phthalic anhydride, toluene, reflux;
b) thionyl chloride, toluene, reflux;
c) N-Bromosuccinimide,CCl4, reflux;
d) quench in MeOH
(Y)= 1 to 5 methylene units

Figure 4; Preparation of Key DOTA Intermediate



a) benzyloxychloroformate, dioxane, H_2O, NaOH
b) $BrCH_2C(O)OC(CH_3)3$, Et_3N, CH_3CN
c) H_2, Pd/C 48 hours, room temp.
d) 2.5 equiv. of 13, excess Et3N, CH3CN
e) 6N HCl, reflux 24 hours
(Y)= 1 to 5 methylene units

Figure 5; Solid Phase Preparation of Amide-DO3A Library



a) H2N(W)NH2, N-Me-Morpholine, THF
b) Bromoacetyl bromide, CH_2Cl_2, diisopropyl ethyl amine
c) 18, CH_2Cl_2, diisopropyl ethyl amine
d) CH_2Cl_2, diisopropyl ethyl amine
) Trifluoroacetic acid, CH2Cl2
(X)= variable spacer unit
(W)= variable spacer unit

GRANT NUMBER
[redacted]

CHECKLIST

1. PROGRAM INCOME *(See Instructions.)*
All applications must indicate whether program inc me is anticipated during the period(s) for which grant support is requested. If program incom is anticipated, use the format below t reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	NONE	

2. ASSURANCES/CERTIFICATIONS *(See instructions.)*
The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/ certifications are provided in Section III of the PHS 398. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

•Human Subjects •Research Using Human Embryonic Stem Cells •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy• Vertebrate Animals

•Debarment and Suspension •Drug- Free Workplace *(applicable to new [Type 1] or revised [Type 1] applications only)*; •Lobbying •Non-Delinquency on Federal Debt •Research Misconduct •Civil Rights (Form HHS 441 or HHS 690); •Handicapped Individuals (Form HHS 641 or HHS 690) •Sex Discrimination (Form HHS 639-A or HHS 690) •Age Discrimination (Form HHS 680 or HHS 690); •Recombinant DNA and Human Gene Transfer Research •Financial Conflict of Interest (except Phase I SBIR/STTR)
•STTR ONLY: Certification of Research Institution Participation.

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicat the applicant organization's most recent F&A cost rate established with th appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will *not* be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Busin ss Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☐ DHHS Agreement dated: ______________________ ☐ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with ______________________ Date __________

CALCULATION*

Entire proposed budget period: Amount of base $ __________ x Rate applied ________ % = F&A costs $ __________
Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☐ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*: No Funds Requested

Principal Investigator/Program Director (Last, first, middle): Garlich, Joseph R.

PERSONNEL REPORT

GRANT NUMBER [redacted]

Place this form at the end of the signed original copy of the application. Do not duplicate.

All Key Personnel for th Current Budget Period

Name	Degree(s)	SSN	R le on Project *(e.g. PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Joseph R. Garlich	Ph.D.	499-50-2700	PI	03/06/56	40.0
Mark Green	Ph.D.	308-68-1357	Subcontract PI	09-10-56	10.0
Alfred C. Dumuaul	Ph.D.	317-88-7551	Post-Doc	05/14/68	100.0

OMB No. 0925-0195
Expiration Date 8/31/2001

Department of Health and Human Services
Public Health Service

Small Business Technology Transfer Program

Phas I Grant Application

Follow instructions carefully.

Leave blank — for PHS use only.		
Type	Activity	Number
Review Group		Formerly
Council Board *(Month, year)*		Date Received

1. TITLE OF APPLICATION *(Do not exceed 56 typewriter spaces)*
Chelate Based Scaffolds (Chelabody) In Tumor Targeting

2. SOLICITATION NO. PHS 2000-2 PAR-01-091 F.L.A.I.R.

3. PRINCIPAL INVESTIGATOR ☐ New Investigator

3a. NAME *(Last, first, middle)*
Joseph R. Garlich

3b. DEGREE(S)
B.A. Ph.D.

[redacted]

3d. POSITION TITLE
Principal Investigator

3e. MAILING ADDRESS *(Street, city, state, zip code)*
9731 Trilobi Drive
Indianapolis, IN 46236

3f. TELEPHONE AND FAX *(Area code, number, and extension)*
TEL: 317-581-1635
FAX: 317-823-7552

BITNET/INTERNET Address:
joegarlich@aol.com

4. HUMAN SUBJECTS
☒ NO
☐ YES

4a. If "Yes," Exemption no. or IRB approval date ☐ Full IRB or ☐ Expedited Review

4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS
☒ NO
☐ YES

5a. If "Yes," IACUC approval date

5b. Animal welfare assurance no.

6. DATES OF PROJECT PERIOD
From: 4/1/02 Through: 3/31/04

7. COSTS REQUESTED
7a. Direct Costs $ $499,369
7b. Total Costs $ 499,369

8. PERFORMANCE SITES *(Organizations and addresses)*
Indiana University; Research and Sponsored Programs
620 Union Drive, Room 618
Indianapolis, IN 46202
Purdue University
1333Pharmacy Building Room 308
West Lafayette, IN 47907-1333
ComChem Technologies, Inc.
8496 Georgetown Road
Indianapolis, IN 46236

9. APPLICANT ORGANIZATION *(Name and address of applicant small business concern)*
ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

10. ENTITY IDENTIFICATION NUMBER #35-2100628
Congressional District 6

11. SMALL BUSINESS CERTIFICATION
☒ Small Business Concern ☐ Women-owned
☐ Socially and Economically Disadvantaged

12. NOTICE OF PROPRIETARY INFORMATION: The information identified by asterisks(*) on pages 17,18,19,20,21,22,23,24 of this application constitutes trade secrets or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that such information shall be used or disclosed only for evaluation of this application, provided that, if a grant is awarded as a result of or in connection with the submission of this application, the Government shall have the right to use or disclose the information herein to the extent provided by law. This restriction does not limit the Government's right t use the information if it is obtained without restriction from another source.

13. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title only of your proposed project, and the name, address, and telephone number of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information or possible investment? ☒ YES ☐ NO

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Barry A. Dreikorn, Ph.D.
Title: Executive Vice President
Address: 9731 Trilobi Drive
Indianapolis, IN 46236

Telephone: 317-823-0732
FAX: 317-823-7552
BITNET/INTERNET Address:
comchemtech@home.com

15. PRINCIPAL INVESTIGATOR ASSURANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a
(In ink. "Per" signature not acceptable.)
Joseph R Garlich

DATE
[redacted]

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF PERSON NAMED IN 14
(In ink. "Per" signature not acceptable.)
Barry Dreikorn

DATE
[redacted]

Abstract of Research Plan

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236
317-823-0732

YEAR FIRM FOUNDED	2000	NO. OF EMPLOYEES (include all affiliates)	3

TITLE OF APPLICATION Chelate Based Scaffolds (Chelabody) In Tumor Targeting

KEY PERSONNEL ENGAGED ON PROJECT

NAME	ORGANIZATION	ROLE ON PROJECT
Joseph R. Garlich, Ph.D.	ComChem Technologies, Inc.	Principal Investigator
TBA	ComChem Technologies, Inc.	Reseach Scientist
TBA, Ph.D.	ComChem Technologies, Inc.	Senior Research Scientist
Mark Green, Ph.D.	Purdue University	Co-Investigator
Carla Mathias	Purdue University	Project Coordinator
TBA, Ph.D.	Purdue University	Post-doc Researcher
Donald L. Durden, M.D.,Ph.D.	Indiana University	Co-Investigator

ABSTRACT OF RESEARCH PLAN: State the application's broad, long-term objectives and specific aims, making reference to the health-relatedness of the project. Describe concisely the research design and methods for achieving these goals and discuss the potential of the research for technological innovation. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. *Therefore, do not include proprietary or confidential information.* DO NOT EXCEED 200 WORDS.

The current paradigm in therapeutic nuclear medicine is to optimize receptor binding molecules and then add on a moiety capable of carrying a radioisotope. This "afterthought" modification process results in suboptimum performance for such agents when dealing with molecules smaller than monoclonal antibodies.

A new concept proposed here is to utilize the properties of chelating agents to build in the desired recognition functionalities. The conformationally restricted metal-ligand complexes proposed herein offer the opportunity to attach molecular recognition units in a certain three-dimensional spatial arrangement that will allow the molecule to mimic protein-protein (or peptide-receptor) binding interactions such as those found in antibody-antigen recognition.

Synthetic molecules that mimic antibody-antigen recognition are known as chemobodies. The new approach in this proposal gives rise to a subset of chemobody molecules hereby termed chelabodies to reflect the critical role that the conformationally restricted metal-ligand complex plays in creating the molecular recognition event.

This concept presented here is broadly applicable to receptors in general but will focus on designing (molecular modeling), synthesizing (through combinatorial methodology), screening (*in vitro*) and optimizing metal-ligand complex-based antagonists of the $\alpha_v\beta_3$ receptor that will deliver therapeutic radioactive metal ions to the neovasculature of $\alpha_v\beta_3$ receptor-positive cancers.

Provide key words (8 maximum) to identify the research or technology.

Combinatorial, chelabody, anticancer , complex, chelating agents, integrins, radiotherapy

Provide a brief summary of the potential commercial applications of the research.

The proposed work is aimed at the discovery, optimization and initial development of a tumor localizing therapeutic radiopharmaceutical drug that targets $\alpha_v\beta_3$ receptors in new blood vessels required for tumor growth. The methodology proposed (combinatorial chelating agent synthesis methodology) is likely to be broadly applicable to address targeting other cell surface receptors.

Year 1 Principal Investigator (Last, first, middle): Garlich, Joseph R.

Budget of Applicant Organization for Phase I—Direct Costs Only	FROM	TO
	[redacted]	[redacted]

PERSONNEL (Applicant organization only)		Type Appt. (months)	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	Role on Project				Salary Requested	Fringe Benefits	TOTALS
Joseph R. Garlich, Ph.D.	P.I	12	15	120,000	0	0	0
TBA, M.S.	Research Scientist	12	100	72,000	72,000	14,400	86,400
TBA, Ph.D	Senior Res. Scientist	12	15	96,000	14,400	2,880	17,280
SUBTOTALS →					86,400	17,280	103,680

CONSULTANT COSTS	
EQUIPMENT (Itemize)	
SUPPLIES (Itemize by category) $13,000 Chemicals and combinatorial chemistry supplies $ 3,000 Glassware	16,000
TRAVEL	
PATIENT CARE COSTS — Inpatient	
PATIENT CARE COSTS — Outpatient	
CONTRACTUAL COSTS Subcontract with Dr. Mark Green of Purdue University for $108,631 Subcontract with Dr. Don Durden of Indiana University for $21,000 (see budget numbers in Dr. Durden's support letter) Total contract costs = $108,631 + $21,000 = $129,671	$129,671
OTHER EXPENSES (Itemize by category)	
TOTAL DIRECT COSTS (Also enter on Face Page, Item 7a) →	$ 249,351
FIXED FEE REQUESTED	$ 0

OTHER SUPPORT (see instructions) [X] NO [ ] YES

 Principal Investigator *(Last, first, middle)*: Garlich, Joseph R.

Budget of Applicant Organization for Phase I—Direct Costs Only

FROM [redacted] TO [redacted]

PERSONNEL *(Applicant organization only)* NAME	Role on Project	Type Appt. *(months)*	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED *(omit cents)* Salary Requested	Fringe Benefits	TOTALS
Joseph R. Garlich, Ph.D.	P.I	12	15	127,200	0	0	0
TBA, M.S.	Research Scientist	12	100	76,320	76,320	15,264	91,584
TBA, Ph.D	Senior Res. Scientist	12	25	101,760	10,176	2,035	12,211
SUBTOTALS →					86,496	17,299	103,795

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*	\$12,500 Chemicals and combinatorial chemistry supplies \$ 2,500 Glassware	15,000
TRAVEL		
PATIENT CARE COSTS	Inpatient Outpatient	
CONTRACTUAL COSTS	Subcontract with Dr. Mark Green of Purdue University for \$113,222 Subcontract with Dr. Don Durden of Indiana University for \$18,001 (see budget numbers in Dr. Durden's support letter) Total contract costs = \$113,222 + \$18,001 = \$131,223	\$113,223
OTHER EXPENSES *(Itemize by category)*		
TOTAL DIRECT COSTS *(Also enter on Face Page, Item 7a)* →		\$250,018
FIXED FEE REQUESTED		\$0

OTHER SUPPORT *(see instructions)* ☒ NO ☐ YES

Principal Investigator (Last, first, middle): Garlich, J seph R.

	FROM:	TO:
Budget of Research Institution for Phase I	[redacted]	[redacted]

NAME AND ADDRESS OF RESEARCH INSTITUTION
Purdue University, West Lafayette, IN 47907

PERSONNEL

NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	Salary Requested	Fringe Benefits	TOTALS
Mark Green, Ph.D.	P.I.	12	10	110,600	11,060	3,235	14,295
IBA, Ph.D.	Post-doc Researcher	12	100	30,000	30,000	5,700	35,700
Carla Mathias	Project Coordinator	12	5	61,900	3,095	639	3,734
SUBTOTALS					44,155	9,574	53,729

CONSULTANT COSTS	
EQUIPMENT (itemize)	
SUPPLIES (itemize by category) $8,718 - Isotope procurement $10,000 - Assay costs, disposables, solvents, counting supplies	18,718
TRAVEL	
PATIENT CARE COSTS — Inpatient	
PATIENT CARE COSTS — Outpatient	
CONTRACTUAL COSTS	
OTHER EXPENSES (itemize by category)	
TOTAL DIRECT COSTS	$ 72,447
INDIRECT COSTS (show calculation) $72,447 x 0.50 = $36,224	36,224
TOTAL COSTS (Also enter as "Contractual Costs" on Budget of Applicant Organization—form page 3)	$108,671

CERTIFICATION OF RESEARCH INSTITUTION PARTICIPATION

Through the signature below of the duly authorized representative of the research institution on this budget page, and by way of the signature of the official signing for applicant organization (small business concern) on the Face Page of the application, the small business concern and the research institution certify jointly that: (1) the proposed STTR project will be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("cooperative research and development"); (2) the proposed STTR project is a cooperative research or research and development effort to be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("performance of research and analytical work"); and (3) regardless of the proportion of the proposed project to be performed by each party, the small business concern will be the primary party that will exercise management direction and control of the performance of the project. If the research institution is a contractor-operated federally funded research and development center, the duly authorized representative of the contractor-operated federally funded research and development center certifies, additionally, that it: (4) is free from organizational conflicts of interests relative to the STTR program; (5) did not use privileged information gained through work performed for an STTR agency or private access to STTR agency personnel in the development of this STTR grant application; and (6) used outside peer review, as appropriate, to evaluate the proposed project and its performance therein.

SIGNATURE of duly authorized representative	Printed Name	Title	Date
	Diane Troyer	Assistant Director	[redacted]

PHS 6246-3 (Rev. 1/93)

Principal Investigator (Last, first, middle): Garlich, Joseph R.

Budget for Research Institution for Phase I	FROM: [redacted]	TO: [redacted]

NAME AND ADDRESS OF RESEARCH INSTITUTION
Purdue University, West Lafayette, IN 47907

PERSONNEL NAME	Role on Project	Type Appt. (months)	% Effort on Project	Institutional Base Salary	Salary Requested	Fringe Benefits	TOTALS
Mark A. Green, Ph.D.	P.I.	12	10	116,130	11,613	3,397	15,010
TBA, Ph.D.	Post-doc Researcher	12	100	31,800	31,800	6,042	37,842
Carla Mathias	Project Coordinator	12	5	64,380	3,219	665	3,884
SUBTOTALS					46,632	10,104	56,736

CONSULTANT COSTS	
EQUIPMENT (Itemize)	
SUPPLIES (Itemize by category) $8,745 - Isotope procurement $10,000 - Assay costs, disposables, solvents, counting supplies	18,745
TRAVEL	
PATIENT CARE COSTS — Inpatient / Outpatient	
CONTRACTUAL COSTS	
OTHER EXPENSES (Itemize by category)	
TOTAL DIRECT COSTS	$ 75,481
INDIRECT COSTS (show calculation) $75,481 x 0.50 = $37,741	37,741
TOTAL COSTS (Also enter as "Contractual Costs" on Budget of Applicant Organization—form page 3)	$113,222

CERTIFICATION OF RESEARCH INSTITUTION PARTICIPATION

Through the signature below of the duly authorized representative of the research institution on this budget page, and by way of the signature of the official signing for applicant organization (small business concern) on the Face Page of the application, the small business concern and the research institution certify *jointly* that: (1) the proposed STTR project will be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("cooperative research and development"); (2) the proposed STTR project is a cooperative research or research and development effort to be conducted jointly by the small business concern and the research institution in which not less than 40 percent of the work will be performed by the small business concern and not less than 30 percent of the work will be performed by the research institution ("performance of research and analytical work"); and (3) regardless of the proportion of the proposed project to be performed by each party, the small business concern will be the primary party that will exercise management direction and control of the performance of the project. If the research institution is a contractor-operated federally funded research and development center, the duly authorized representative of the contractor-operated federally funded research and development center certifies, additionally, that it: (4) is free from organizational conflicts of interests relative to the STTR program; (5) did not use privileged information gained through work performed for an STTR agency or private access to STTR agency personnel in the development of this STTR grant application; and (6) used outside peer review, as appropriate, to evaluate the proposed project and its performance therein.

SIGNATURE of duly authorized representative	Printed Name	Title	Date
D. Troyer	Diane Troyer	Assistant Director	[redacted]

PHS 6246-? (Rev. 1/98)

Page 4

Budget Justification

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify costs accordingly.

Applicant Organization Pers nnel:

Joseph R. Garlich, Ph.D., Principal Investigator, will contribute 15% of his time (and no salary as his compensation be leverage money supplied by CCTI) and will assist in the experimental design and implementation of synthetic work, both traditional and combinatorial (solid-phase) and supervise and coordinate the experimental studies. Responsible jointly with Dr. Green/Dr. Durden for interpretation of the data and providing project direction.

TBA, Ph.D., Senior Research Associate., will be skilled in organic synthesis (solution and solid phase) and have molecular modeling expertise. This position will contribute 15% of time to the project performing hands-on solid phase synthesis, experimental design, and molecular modeling studies.

TBA, M.S., Research Associate, will be skilled in organic synthesis (solution and solid phase) with some experience in complexation chemistry and will be well versed in analytical instrumentation and purification methods. Will be responsible for developing solid phase protocols and production and purification of combinatorial libraries of target molecules.

Research Institutions Personnel:

Donald Durden, M.D., Ph.D., Co-Investigator at Indiana University will assist in designing $\alpha v\beta 3$ bioassays, interpretation of results, biochemical pathways, and serve as an expert on vascular biology. Although his time commitment (7%) is modest the expertise he brings to the biological assessment is critical.

Dr. Mark Green, Ph.D., Co-Investigator at Purdue University, will contribute 10% of his time in the experimental design of the project including the chelator design, bioassays, radioisotope labeling, and interpretation of the experimental results.

TBA, Ph.D., Post-Doc researcher at Purdue will conduct the biochemistry and medium-throughput bioassays, perform experimental design, data collection, presentation and interpretation.

Carla Mathias, B.A., Project Coordinator at Purdue, will serve, by benefit of extensive radiopharmaceutical laboratory experience, as coordinator and participant in the design and implementation of the radiochemistry studies including complexation and analyses thereof.

Resources

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. (The research to be performed by the applicant small business concern and its collaborators must be in facilities that are available to and under the control of each party for the conduct of each party's portion of the proposed project.) Indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant small business concern and any other performance site listed on the FACE PAGE. Identify support services such as secretarial, machine shop, electronics shop, and the extent to which they will be available to the project. Use continuation page(s) if necessary.

Research Institution: Purdue University (West Lafayette, Indiana) is less than a 2 hour drive from the Indianapolis facility of CCTI. Major shared instrumentation and facilities are available within the School of Pharmacy. The Combinatorial Chemical Biology Center is in the same building and will be a resource for the biological screening. In Dr. Green's research laboratories support equipment includes automatic counting systems, TLC radiography equipment and HPLC systems with radiometric detectors. Dr. Durden's research laboratory is in the Cancer Research Institute located at Indiana University in Indianapolis, a 20 minute drive from CCTI. His equipment available for this project includes molecular and cell biology equipment such as ultracentrifuges, scintillation counters, gamma counter, gel dryers, incubators, and flow analysis instrumentation.

Applicant Organization: ComChem Technologies (CCTI) will have in place at its facility standard synthesis equipment but more importantly will have equipment for combinatorial chemical synthesis (solution and solid-phase), both protocol development and library production tools (parallel reaction equipment, automated LCMS, software, liquid handler, etc.).

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each. **Research Institutions:** NMR (multinuclear Varian VXR-500 MHz, Bruker ARX 300 MHz); Mass Spect (MAT L95 HRMS, Finnigan 4000 for EI/CI, and Thermoquest LCQ with electrospray and LC/MS/MS); Beckman DU7-HS U.V. spectrometer; Nicolet FT-IR; and several scintillation counters. The Combinatorial Chemical Biology Center houses a Tecan Spectrafluor Plus, BioImage Intelligent Quantifier (IQ) for blot analysis and colony counting, PDI Discovery Series Scanner.Densitomer, Beckman LS1801 beta counter, Packard microplate scintillation and luminescence counter, and Molecular Dynamics STORM Phosphorimager. **Applicant Org.:** CCTI has Shimadzu preparatory and analytical HPLC-MS systems with ESI/APCI capability and evaporative light scattering detector.

Joseph R. Garlich

President and Chief Scientific Officer
ComChem Technologies Inc.

Education:

Institute and Location	Degree	Year(s)	Field of Study
University of Missouri, Columbia, MO	BA	1974-78	Chemistry
University of Missouri, Columbia, MO	BA	1974-78	Biology
University of Missouri, Columbia, MO	Ph.D.	1978-82	Organic Chemistry
University of Florida, Gainesville, FL	(Post-Doc)	1982-84	Medicinal Chemistry

Professional Experience:

2000-present President, founder, and Chief Scientist of ComChem Technologies, Inc., Indianapolis, IN. Drug discovery/development using combinatorial chemistry.

1997-2000 Research Scientist, Combinatorial Chemistry-Lead Generation, DowAgroSciences, Indianapolis, IN.

1995-1997 Research Scientist, Discovery Research Department, DowElanco, Indianapolis, IN.

1993-1995 Research Associate, Designed Chemicals R & D Department, Dow Chemical Company, Freeport, TX.

1990-1993 Research Leader, Designed Chemicals R & D Department, Dow Chemical Company, Freeport, TX.

1987-1990 Project Leader, Functional Chemicals Research Department, Dow Chemical Company, Freeport, TX.

1984-1987 Senior Research Chemist, Organic Process Research Department, Dow Chemical Company, Freeport, TX.

Honors and Awards:

1992 Gulf Coast Scientists Texas Inventor of the Year Award, Dow Chemical

1992 Gulf Coast Scientists Award For Excellence in Science, Dow Chemical

1997 DowElanco Discovery Recognition Award for Excellence in Problem Solving

SELECTED BIBLIOGRAPHY:

DeAmicis, C.V., Dripps, J.E., Garlich, J.R., Hatton, C.H., Hill, R.L. "Photochemical Stability of Spinosad and Semi-synthetic Spinosyn Derivatives" J. Agr. Food Chem. Submitted 2001.

Crouse, G.D., Sparks, T.G., Schoonover, J., Gifford, J., Dripps, J., Bruce, T., Larson, L.L., Garlich, J., Hatton, C., Hill, R.L., Worden, T.V., Martynow, J.G. "Recent Advances in the Chemistry of Spinosyns", Pest Management Science, in press, 2001.

Kleschick, W.A., Davis, L.N., Dick, M.R., Garlich, J.R., Martin, E.J., Orr, N.,Ng, S.C., Pernich, D.J., Unger, S.H., Watson, G.B., Zuckermann, R.N., "The Application of Combinatorial Chemistry in Agrochemical Discovery", ACS Symposium Series 774; Agrochemical Discovery; pp 205-213, 2001.

Cooper, D.H, Garlich, J.R., Ritzler, S. "Solution Phase Parallel Synthesis of a 1408 Member Library of Phosphonic Acids and Esters, Poster presented at the 1st Annual Indiana ACS Poster Session, Indianapolis, Indiana, October 9, 2000.

Garlich, J.R., Ritzler, S.J. "Novel Nucleophilic Cleavage Agents", Poster presented at the 5th Annual High Throughput Synthesis Symposium, San Diego, CA., February 11, 2000.

Invited Seminar, IUPUI Department of Chemistry, "Combinatorial Chemistry Applications in Agrochemical Discovery", January 26, 2000.

Garlich, J.R. , "Studies and Analogs of a Triglycine Lead Molecule", poster presented to the 37th National Organic Chemistry Symposium, Madison, Wisconson, June 14, 1999.

Bayouth, J., Macey, D., Kasi, L., Garlich, J., McMillan, K., Dimopoulos, M., Champlin, R., "Pharmacokinetics, Dosimetry and Toxicity of Holmium-166-DOTMP for Bone Marrow Ablation in Multiple Myeloma", Journal of Nuclear Medicine, Volume 36, pp. 730-737, 1995.

Champlin, R., Dimopoulos, M., Bayouth, J., Macey, D., Kasi L., Przepiorka, D., Pololoff, D., Garlich, J., Simon, J., Alexanian, R. "Holmium-166 DOTMP, A Bone Seeking Radiochelate For Selective Marrow Radiotherapy With Bone Marrow Transplantation (BMT) For Multiple Myeloma", presented by Dr. Champlin at the International Society of Experimental Hematology, Rotterdam, September 1993.

Ghiron, J., Volkert, W.A., Garlich, JR., "Determination of Lesion to Normal Bone Uptake Ratios of Skeletal Radiopharmaceuticals by QARG", Nuclear Medicine and Biology, Volume 18, pp. 235-240, 1991.

Parks, N.J., Kawakami, T.G., Homoff, W., Fisher, P., Garlich, J.R., Simon, J., and Champlin, R., "Bone Marrow Transplantation in Dogs After Radioablation with a Ho-166 Amino Phosphonic Acid Bone-Seeking Agent (DOTMP) ",Blood, Volume 82, pp 318-325, 1993.

Garlich, J.R., " ^{166}Ho-DOTMP: A New Agent For Bone Marrow Ablation" Presented at the Fortieth Annual Meeting of the Society of Nuclear Medicine, June 8, 1993, Toronto, Canada.

Garlich, J.R., "Chemistry of Novel Macrocyclic Aminophosphonic Acid Chelates of Rare Earth Radionuclides and Their In-Vivo Biodistribution". Presented at the Fortieth Annual Meeting of the Society of Nuclear Medicine, June 8, 1993, Toronto, Canada.

ISSUED UNITED STATES PATENTS:

1. Bone Marrow Suppressing Agents 4,882,142 (11/21/89)
2. Method For Purifying Aminomethylenephosphonic Acids for Pharmaceutical Use. 4,937,333 (6/26/90)
3. Bone Marrow Suppressing Agents. 4,976,950 (12/11/90)
4. Macrocyclic Aminophosphonic Acid Complexes For the Treatment of Calcific Tumors. 5,059,412 (10/22/91)
5. Macrocyclic Aminophosphonic Acid Complexes, Their Formulations and Use. 5,064,633 (11/12/91)
6. Radiolabeled Metal-Binding Protein for the Treatment of Arthritis. 5,133,956 (7/28/92)
7. Oral Compositions for Suppressing Mouth Odors. 5,286,479 (2/15/94)
8. Organic Amine Phosphonic Acid Complexes for the Treatment of Calcific Tumors. 5,300,279 (4/5/94)
9. Phytate Antimicrobial Compositions in Oral Care Products. 5,300,289 (4/5/94)
10. Method of Treating and/or Diagnosing Soft Tissue Tumors. 5,308,606 (5/3/94)
11. Oral Compositions for Inhibiting Calculus Formation. 5,318,772 (6/7/94)
12. Oral Compositions for Inhibiting Plaque Formation. 5,320,829 (6/14/94)
13. Complexes Possessing Ortho Ligating Functionality. 5,342,604 (8/30/94)
14. Radioactive Compositions for Soft Tissue Tumors. 5,342,925 (8/30/94)
15. Macrocyclic Conjugates and Their Use as Diagnostic and Therapeutic Agents. 5,435,990 (7/25/95)
16. Macrocyclic Ligands and Complexes. 5,652,361 (7/29/97)
17. Complexes Possessing Ortho Ligating Functionality and Complexes Thereof. 5,696,239 (12/9/97)
18. Conjugates Possessing Ortho Ligating Functionality. 5,714,631 (2/3/98)
19. Bicyclopolyazamacrocyclophosphonic Acid Complexes for use as Contrast Agents. 5,739,294 (4/14/98)
20. Bicyclopolyazamacrocyclophosphonic Acid Half Esters. 5,750,660 (5/12/98)
21. Macrocyclic Tetraazacyclododecane Conjugates and Their Use as Diagnostic and Therapeutic Agents. 5,756,065 (5/26/98)
22. Frozen Radiopharmaceutical Formulations. 5,762,907 (6/9/98)

PUBLISHED PENDING FOREIGN PATENT APPLICATIONS:

1. Carbonyl-Containing Degradable Chelants, Uses, and Compositions Thereof (EP-522547-A2; 1/13/93).
2. Targeted Delivery of Growth Factors for Bone Regeneration (PCT Int. Appl. WO 94/00145, 1/6/94).
3. Bicyclopolyazamacrocyclophosphonic Acids, Their Complexes and Conjugates, for use as Contrast Agents, and Processes for their Preparation (WO 94/26754. 11/24/94).

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Mark A. Green	Professor f Medicinal Chemistry

EDUCATION (Begin with baccalaureate or other initial professional education,. Include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Rose-Hulman Institute of Technology, Terre Haute, Indiana	B.S.	1978	Chemistry
Indiana University, Bloomington, Indiana	Ph.D.	1982	Inorganic Chemistry
Washington University, St. Louis, Missouri	Postdoctoral	1982-85	Radiopharmaceutical Chem.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to those publications most pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Positions:

9/78-8/82 Associate Instructor and Research Associate, Department of Chemistry, Indiana University, Bloomington, IN. Research advisor: Professor Kenneth G. Caulton.

8/82-6/85 Postdoctoral Research Associate with Professor Michael J. Welch, Department of Radiology, Washington University School of Medicine, St. Louis, Missouri.

7/85-7/87 Assistant Professor, Department of Radiology, University of Minnesota Medical School, Minneapolis, Minnesota. Joint appointment, College of Pharmacy, Department of Medicinal Chemistry.

7/87-6/90 Assistant Professor of Nuclear Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.

3/90-present Adjunct Faculty Appointment, Department of Radiology, Indiana University School of Medicine, Indianapolis, Indiana.

7/90-6/94 Associate Professor of Medicinal Chemistry, Division of Nuclear Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.

7/94-present Professor of Medicinal Chemistry, Division of Nuclear Pharmacy, Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.

Awards and Other Professional Activities:

Twelfth Tetalman Memorial Award, The Society of Nuclear Medicine, 1992
NIH Research Career Development Award, from the National Heart, Lung, and Blood Institute, 8/86-7/91; Tau Beta Pi, 1977
American Chemical Society, 1977-present; Society of Nuclear Medicine, 1983-present; Sigma Xi, 1988-present;
International Society of Cerebral Blood Flow and Metabolism, 1991-present; Institute for Clinical PET, 1991-present
American Association for Cancer Research, 1997-present. Society for Nuclear Imaging in Drug Development, 2000-present.

Most Recent Publications Relevant To This Proposal (from a total of 92):

"Synthesis of Compound Libraries Based on 3,4-Diaminocyclopentanol Scaffolds," *J. Comb. Chem.*, 2:297-300; 2000. Y. Guan, M.A. Green, and D.E. Bergstrom.

"Novel gallium(III) complexes transported by MDR1 P-glycoprotein: potential PET imaging agents for probing P-glycoprotein-mediated transport activity *in vivo*," *Chemistry and Biology*, 7:335-343; 2000. V. Sharma, A. Beatty, S.P. Wey, L. Bass, C.L. Crankshaw, M.A. Green, M.J. Welch, and D. Piwnica-Worms.

"Synthesis of [^{99m}Tc]-Tc-DTPA-Folate and Its Evaluation as a Folate-Receptor-Targeted Radiopharmaceutical," *Bioconjugate Chemistry* 11:253-257; 2000. C.J. Mathias, D. Hubers, P.S. Low, and M.A. Green.

"A Kit Formulation for Preparation of [^{111}In]In-DTPA-Folate, a Folate-Receptor-Targeted Radiopharmaceutical," *Nucl. Med. Biol.*, 25:585-587; 1998. C.J. Mathias and M.A. Green.

"Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts," *Nucl. Med. Biol.*, 26:23-25; 1999. C.J. Mathias, S. Wang, P.S. Low, D.J. Waters, and M.A. Green.

"Evaluation of ^{111}In-DTPA-Folate as a Potential Folate-Receptor-Targeted Radiopharmaceutical," *J. Nucl. Med.*, 39:1579-1585; 1998. C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, and M.A. Green.

"Structure-Activity Relationships for Metal-Labeled Blood Flow Tracers: Comparison of Ketoaldehyde *Bis*(thiosemicarbazonat)copper(II) Derivatives," *J. Med Chem.*, 33, 1764-1770, 1990. E.K. John and M.A. Green.

"Investigation of Cu-PTSM as a PET Tracer for Tumor Blood Flow," *Nucl. Med. Biol.*, 18, 807-811, 1991. C.J. Mathias, M.J. Welch, D.J. Perry, A.H. McGuire, X. Zhu, J.M. Connett, and M.A. Green.

"PET Imaging with Metal Radionuclides" in *Advances in Metals in Medicine, Volume 1*, M.J. Abrams and B.A. Murrer, editors, JAI Press, Greenwich, CT, 1993, pages 75 - 114. M.A. Green.

"Subcellular Distribution of Tissue Radiocopper Following Intravenous Administration of ^{67}Cu-Labeled Cu-PTSM." *Nucl. Med. Biol.*, 19, 697-701, 1992. I.D. Baerga, R.P. Maickel, and M.A. Green.

"Potential Gallium-68 Tracers for Imaging the Heart with Positron Emission Tomography: Evaluation of Four Gallium Complexes with Functionalized Tripodal *Tris*(salicylaldimine) Ligands." *J. Nucl. Med.*, 34, 228-233, 1993. M.A. Green, C.J. Mathias, W.L. Neumann, M.Janik, and E.A. Deutsch.

"Quantification of Regional Myocardial Perfusion with Generator-Produced Copper-62-PTSM and Positron Emission Tomography." *Circulation*, 87, 173-183, 1993. P. Herrero, J. Markham, C.J. Weinheimer, C.J. Anderson, M.J. Welch, M.A. Green, and S.R. Bergmann.

"Development and Validation of a Solvent Extraction Technique for Determination of Cu-PTSM in Blood." *Nucl. Med. Biol.*, 20, 343-349, 1993. C. J. Mathias, S.R. Bergmann, and M.A. Green.

"A Gallium-68 Radiopharmaceutical that is Retained in Myocardium: ^{68}Ga[4,6-MeO$_2$sal)$_2$BAPEN]$^+$." *J. Nucl. Med.*, 34:1127 - 1131, 1993. B.W. Tsang, C.J. Mathias, and M.A. Green.

"Synthesis and Structure of a Five Coordinate Triarylgallium Complex. *J. Chem. Soc., Chem. Comm.*, 14, 1127-1129, 1993. D.K. Coggin, P.E. Fanwick, and M.A. Green.

"Evaluation of Cu-PTSM as a Tracer of Tumor Perfusion: Comparison with Labeled Microspheres in Spontaneous Canine Neoplasms." *Nucl. Med. Biol.*, 21, 83 - 87, 1994. C. J. Mathias, M. A. Green, W. B. Morrison, and D. W. Knapp.

"Structure-Distribution Relationships for Metal-Labeled Myocardial Imaging Agents: Comparison of a Series of Cationic Ga(III) Complexes with Hexadentate Bis(salicylaldimine) Ligands." *J. Med. Chem.*, 37:4400-4406, 1994. B.W. Tsang, C.J. Mathias, P.E. Fanwick, and M.A. Green.

"Species-Dependent Binding of Copper(II) *Bis*(thiosemicarbazone) Radiopharmaceuticals to Serum Albumin." *J. Nucl. Med.*, 36: 1451-1455, 1995. C.J. Mathias, S.R. Bergmann, and M.A. Green.

"Synthesis, Purification, and Tumor Cell Uptake of ^{67}Ga-Deferoxamine-Folate Conjugate, a Potential Radiopharmaceutical for Tumor Imaging." *Bioconjujate Chemistry*, 7:56-62; 1996. S. Wang, R.J. Lee, C.J. Mathias, M.A. Green, and P.S. Low.

"Tumor-Selective Radiopharmaceutical targeting via Receptor-Mediated Endocytosis: Evaluation of a Gallium-67 Labeled Folate-Deferoxamine Conjugate." *J. Nucl. Med.*, 37:1003-1008; 1996. C.J. Mathias, S. Wang, R.J. Lee, D.J. Waters, P.S. Low, and M.A. Green.

"Assessment of Regional Myocardial Perfusion with Generator-Produced ^{62}Cu-PTSM and PET in Human Subjects." *J. Nucl. Med.*, 37:1294-1300; 1996. P. Herrero, J.J. Hartman, M.A. Green, C.J. Anderson, M.J. Welch, J. Markham, and S.R. Bergmann.

"Mixed bis(thiosemicarbazone) ligands for the preparation of copper radiopharmaceuticals: synthesis and evaluation of tetradentate ligands containing two dissimilar thiosemicarbazone functions.: *J. Med. Chem.*, 40:132-136; 1997. J.K. Lim, C.J. Mathias, and M.A. Green.

"Design and Synthesis of ^{111}In-DTPA-Folate for Use as a Tumor-Targeted Radiopharmaceutical," *Bioconj. Chem.*, 8:673-679; 1997. S. Wang, J. Luo, D.A. Lantrip, D.J. Waters, C.J. Mathias, M.A. Green, P.L. Fuchs, and P.S. Low.

"Evaluation of ^{111}In-DTPA-Folate as a Potential Folate-Receptor-Targeted Radiopharmaceutical," *J. Nucl. Med.*, 39:1579-1585; 1998. C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, and M.A. Green.

"Human Biodistribution and Dosimetry of the PET Perfusion Agent ^{62}Cu-PTSM from a Compact Modular ^{62}Zn/^{62}Cu Generator," *J. Nucl. Med.*, 39:1958-1964; 1998. T.R. Wallhaus, J. Lacy, J. Whang, M.A. Green, R.J. Nickles, and C.K. Stone.

"Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts," *Nucl. Med. Biol.*, 26:23-25; 1999. C.J. Mathias, S. Wang, P.S. Low, D.J. Waters, and M.A. Green.

"A Kit Formulation for Preparation of [^{111}In]In-DTPA-Folate, a Folate-Receptor-Targeted Radiopharmaceutical," *Nucl. Med. Biol.*, 25:585-587; 1998. C.J. Mathias and M.A. Green.

"Stereocontrolled Synthesis of (*R,R,S*)- and (*S,R,S*)-3,4-Diaminocyclopentanols." *SYNLETT* 1999:426-428. Y. Guan, D.E. Bergstrom, and M.A. Green.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Carla J. Mathias	Project Coordinator

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
DePauw University, Greencastle, Indiana	B.A.	1976	Zoology & Chemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

Professional Positions:

12/77 - 10/78 Research Technician I, Hemostasis and Thrombosis Research, with H. J. Joist, M.D., Washington University School of Medicine, St. Louis, Missouri.

7/78 - 6/86 Senior Research Technician, Nuclear Medicine Research, with M. J. Welch, Ph.D. and B. A. Siegel, M.D., Washington University School of Medicine, St. Louis, Missouri.

7/86 - 6/89 Research Assistant, Division of Radiation Sciences, with M. J. Welch, Ph.D., Washington University School of Medicine, St. Louis, Missouri.

7/89 - 6/90 Research Associate, Division of Radiation Sciences, with M. J. Welch, Ph.D., Washington University School of Medicine, St. Louis, Missouri

1/91 - 8/94 Visiting Research Instructor, Department of Medicinal Chemistry, School of Pharmacy, Purdue University, West Lafayette, Indiana

7/94 - 6/95 Project Coordinator, Purdue National Biomedical Tracer Facility Project, Purdue University, West Lafayette, Indiana

6/96 - present Research Project Coordinator, Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy, Purdue University, West Lafayette, Indiana

Awards and Other Professional Activities:

Missouri Valley Chapter-Society of Nuclear Medicine, Young Investigator Award, Runner-up, 1979-1981;Young Investigator Award, 1982

National Science Foundation, Travel Award, to N.A.T.O. Advanced Studies Institute, Greece, 6/87

Society of Nuclear Medicine, Berson-Yalow Award (annual award for outstanding paper in the application of radioisotope techniques in receptor or immunoassay), Co-awardee in both 1988 and 1990.

Relevant Publications (selected from a total of 85):

C.J. Mathias, D. Hubers, P.S. Low, and M.A. Green. Synthesis of [^{99m}Tc]-Tc-DTPA-Folate and Its Evaluation as a Folate-Receptor-Targeted Radiopharmaceutical," *Bioconjugate Chemistry* 11:253-257; 2000.

C.J. Mathias and M.A. Green. A Kit Formulation for Preparation of [^{111}In]In-DTPA-Folate, a Folate-Receptor-Targeted Radiopharmaceutical, *Nucl. Med. Biol.*, 25:585-587; 1998.

C.J. Mathias, S. Wang, P.S. Low, D.J. Waters, and M.A. Green. Receptor-Mediated Targeting of ^{67}Ga-Deferoxamine-Folate to Folate-Receptor-Positive Human KB Tumor Xenografts, *Nucl. Med. Biol.*, 26:23-25; 1999.

C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, and M.A. Green. Evaluation of ^{111}In-DTPA-Folate as a Potential Folate-Receptor-Targeted Radiopharmaceutical, *J. Nucl. Med.*, 39:1579-1585; 1998.

S. Wang, J. Luo, D.A. Lantrip, D.J. Waters, C.J. Mathias, M.A. Green, P.L. Fuchs, and P.S. Low. Design and Synthesis of ^{111}In-DTPA-Folate for Use as a Tumor-Targeted Radiopharmaceutical, *Bioconj. Chem.*, 8:673-679; 1997.

C.J. Mathias, S. Wang, R.J. Lee, D.J. Waters, P.S. Low, and M.A. Green. Tumor-Selective Radiopharmaceutical Targeting via Receptor-mediated Endocytosis: Evaluation of a Gallium-67 Labeled Folate-Deferoxamine Conjugate. *J. Nucl. Med.*, 37:1003-1008; 1996.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Donald L. Durden, M.D., Ph.D.	Associate Professor of Pediatrics & Biochemistry

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of South Florida, Tampa, FL	B.S.	1977	Microbiology/Zoology
University of Miami School of Medicine, Miami, FL	Ph.D.	1983	Microbiology/Immunology
University of Miami School of Medicine, Miami, FL	M.D.	1985	Medical Doctor
Childrens Hospital of Medical Center, Seattle, WA	Fellow	1987-1988	Pediatric Hem/Onc
Fred Hutchinson Cancer Research Center, Seattle, WA	Fellow	1988-1992	Molecular/Cell Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at th masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1999-Present	Associate Professor, Pediatrics and Biochemistry and Molecular Biology, Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana.
1993-Apr. 1999	Assistant Professor, Division of Hematology-Oncology, Department of Pediatrics, Childrens Hospital Los Angeles/University of Southern California School of Medicine, Los Angeles, California.
1989-1992	Postdoctoral fellowship, Fred Hutchinson Cancer Research Center, Seattle, WA, Role of tyrosine phosphorylation in myeloid signal transduction, Jonathan Cooper, Supervisor.
1979-1985	Graduate/Medical Student Research, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL. Isolation and characterization of Vibrio L-asparaginase. J.A. Distasio, Advisor.

SELECTED PUBLICATIONS:

1. Charyulu, V., Sigel, M.M., **Durden, D.L.**, and Lopez, D.M. Mouse mammary tumor virus (MMTV) antigen(s) are present on B-lymphocytes of Balb/c mice. *Int J Cance,r* 24:813-818, 1979.
2. **Durden, D.L.** and Distasio, J.A. Comparison of the immunosuppressive effects of L-asparaginases from *Escherichia coli* and *Vibrio succinogenes*. *Cancer Res*, 40:1125-1129, 1980.
3. **Durden, D.L.** and Distasio, J.A. Characterization of the effects of *Escherichia coli* asparaginase and a glutaminase-free asparaginase from *Vibrio succinogenes* on specific cell-mediated cytotoxicity. *Int J Cancer*, 27:59-65, 1981.
4. Distasio, J.A., **Durden, D.L.**, Paul, R.D., and Nadji, M. Alteration in spleen lymphoid populations associated with specific amino acid depletion during L-asparaginase treatment. *Cancer Res*, 42:252-258, 1982.
5. Distasio, J.A., Salazar, A.M., Nadji, M., and **Durden, D.L.** Glutaminase-free asparaginase from *Vibrio succinogenes*: an antilymphoma enzyme lacking hepatoxicity. *Int J Cancer*, 30:343-347, 1982.
6. **Durden, D.L.**, Salazar, A.M., and Distasio, J.A. Kinetic analysis of hepatotoxicity associated with antineoplastic asparaginases. *Cancer Res.*, 43:1602-1605, 1983.
7. Kazlauskas, A., **Durden, D.L.**, and Cooper, J.A. Functions of the major tyrosine phosphorylation site of the PDGF receptor beta subunit. *Cell Regul*, 2:413-425, 1991.

8. **Durden, D.L.**, Rosen, H., and Cooper, J.A. Serine/threonine phosphorylation of the □ subunit after activation of the high-affinity Fc receptor for immunoglobulin G. *Biochem, J* 299:569-577, 1994.

9. **Durden, D.L.**, Rosen, H., Michel, B.R., and Cooper, J.A. Protein tyrosine phosphatase inhibitors block myeloid signal transduction through the Fc□RI receptor. *Exp Cell Res,* 211:150-162, 1994.

10. **Durden, D.L.**, and Liu, Y.B. Protein-tyrosine kinase p72^{syk} in Fc□RI receptor signaling. *Blood,* 84:2102-2108 (rapid communication), 1994.

11. **Durden, D.L.**, Kim, H.M., Calore, B., and Liu, Y.B. The Fc□RI receptor signals through the activation of *hck* and MAP kinase. *J Immun,* 154:4039-4047, 1995.

12. Arditi, M., Zhou, J., Martine, T., **Durden, D.L.**, Stins, M., and Kim, K-S. Lipopolysaccharide stimulates the tyrosine phosphorylation of mitogen-activated protein kinases, p44, p42, and p38 in vascular endothelial cells in a soluble CD14-dependent manner: Role of protein tyrosine phosphorylation in lipopolysaccharide-induced stimulation of endothelial cells. *J Immun,* 155(8):3994-4003, 1995.

13. Park, R.K., Liu, Y.B., and **Durden, D.L.** A role for Shc, Grb2 and Raf-1 in Fc□RI signal relay. *J Biol Chem,* 271:13342-13348, 1996.

14. Taylor, N., Jahn, T., Smith, S., Uribe, L., Liu, Y.B., **Durden, D.L.**, and Weinberg, K. Differential activation of the tyrosine kinases ZAP-70 and SYK in Fc□RI signaling. *Blood,* 89:388-396, 1997.

15. Epstein, A., Liu, M., Liu, Y.B. and **Durden, D.L.**, Protein tyrosine phosphtase inhibitors in Fc□RI induced myeloid oxidant signal transducton. *Exp. Cell Res,* 237:288-295, 1997.

16. Park, R.K., Liu, Y.B., Kyono, W., and **Durden, D.L.** CBL-GRB2 adapter protein interaction in immunoreceptor tyrosine activation motif (ITAM) signaling. *J. Immunol,* 160:5018-5027, 1998.

17. Chu, J., Liu, Y, Koretzky, G.A. and **Durden, D.L.** SLP-76-CBL-Grb2-Shc interactions in Fc□RI signaling. *Blood,* 92:1697-1706, 1998.

18. Kyono, W. T., De Jong, R., Park, R. K., Liu, Y.B., Heisterkamp, N., Groffen, J. and **Durden, D.L.** Differential interaction of Crkl with Cbl or C3G, Hef-1 and □-subunit ITAM in myeloid Fc□RI signaling. *J. Immunol,* 161:5555-5563, 1998.

19. Izadi, K., Erdreich-Epstein, A., Liu, Y., and **Durden, D.L.** Characterization of Cbl-Nck and Nck-Pak1 interactions in myeloid Fc□RII signaling. *Exp Cell Res,* 245:330-342, 1998.

20. Erdreich-Epstein, A., Liu, M. Kant, A., Izadi, K., Nolta, J. and **Durden, D.L.** CBL functions downstream of Src kinases in FcγRI signaling in primary human macrophages *J. Leuk. Biol,* 65:523-534, 1999.

22. Park, Rae-Kil, Izadi, K., Deo, Y.M., Liu, Y.B. and **Durden, D.L.** Role of Src in the modulation of multiple adaptor proteins in Fc□RI oxidant signaling. *Blood,* 94:2112-2120, 1999.

23. Park, R.K., Erdreich-Epstein, A., Liu, M., Izadi, K.D., and **Durden, D.L.** High affinity IgG receptor activation of Src family kinases is required for modulation of the Shc-Grb2-Sos complex and the downstream activation of the nicotinamide adenine dinucleotide phosphate (reduced) oxidase. *J. Immunol,* 163:6023-6034, 1999.

24. Erdreich-Epstein, A., Shimada, H., Groshen, S., Liu, M., Metelitsa, L., Kim, K.S., Stins, M., Seeger, R.C. and **Durden, D.L.** Integrins □v□3 and □v□5 are expressed by endothelium of high-risk neuroblastoma and their inhibition is associated with increased endogenous ceramide. *Cancer Research,* 60:712-721, 2000.

25. Wen, S., Stolarov, J., Su, J.D., Wigler, M.H., Tonks, N.K., **Durden, D.L.** PTEN controls tumor-induced angiogenesis. *PNAS.* In Press, 2001.

26. Isogai, C, Laug, W.E., Shimad, H., Declerck, P., Stins, M. Erdreicht Epstein, A., **Durden, D.L.**, De Clerck, Y.A.. PAI-1 Promotes Angiogenesis by Stimulating Endothelial Cell Migration Toward Fibronectin. *Cancer Research.* In Press, 2001.

27. Reif, A., Zastrow, M., Sun, B-C., Takei, S., Misuhada, H. Bernstein, B., **Durden D.L.** Treatment of collagen induced arthritis in DBA/1 mice with L-asparaginase. *Clin Exp Rheumatol.* In Press, 2001.

STTR Resubmission Introductory Page **Principal Investigator:** Garlich, Joseph R.

Based on the reviewers comments and recent developments in the art we have substantially revised our proposal. One major change reflects the bulk of the reviewers concerns about the biological evaluations and that is to bring Dr. Don Durden, M.D.,Ph.D.into the project as a collaborator (versus a limited consultant in the original submission). His documented experience in integrin signaling and in particular with αvβ3 gives us the combined expertise for all aspects of the proposal. Additionally, we have dropped the animal experiments until phase II and focused more specifically on the chemistry aspects (especially library preparation details) of the project. Additions to the proposal are indicated by a vertical line in the right hand margin.

Specific reviewer concerns (paraphrased and bolded) and our remedies are listed below:

1. **Biological evaluation doesn't show appreciation for complexity of integrin receptors and animal experiments are premature:** We have dramatically revamped our biological assay methods to utilize cell lines which expresses αvβ3. This approach is supported by reviewer cited references and recent presentations at the Society of Nuclear Medicine meeting. We have put off animal studies till phase II.
2. **Overly ambitious, need more focus on compound design and synthesis:** More details are provided to help illustrate the targeted compounds and methods to arrive at them.
3. **Is αvβ3 a suitable first target for this chelabody approach and if so should we also look at other integrins:** It is a fine target because significant recent work exists on antagonist structure-activity relationships that gives us a focus for our combinatorial chemistry. Other integrins that we do not want to bind with or antagonize are being looked at in the new assays from a negative control standpoint.
4. **Receptor-ligand washing protocol and internalization studies are ill defined and will compounds be compared against gold standard such as c(RGDfK)?** The bioassay protocols are now much more defined and all of the assays will include the gold standard c(RDGfk) for interassay comparison, for competition experiments.
5. **Is there enough resources for the laborious synthesis?** This is the beauty of combinatorial sythesis and parallel synthesis techniques which Dr. Garlich is in a position to assess in his former postition as leader for Combichem at Dow AgroSciences.
6. **Where is expertise in bioassays?** We have brought Dr. Durden, an expert in bioassays, cell signaling, and integrins, into this proposal as a collaborator to ensure that our bioassays will yield relevant useful data.
7. **Little info provided on composition of libraries, molecular modeling has many shortfalls, and is the 1000 compounds proposed enough to probe a substantial amount of chemical space?** We have embellished the nature of the substituents that define the library composition. Molecular modeling is a rough tool that simply points us in the right direction. The power of combinatorial chemistry is used to overcome the inaccuracies of molecular modeling to find the right set of binders. The proposed 1000 compounds is not a hard number but an illustrative one. We plan to prepare a set of compounds, the number of which willl be determined by how well the various chemistries work, and then perform bioassays. The bioassay result, in conjunctiton with modeling will help refine the next set of compounds to prepare. In this manner, chemical space is probed and then we follow up by zeroing in on the bioactive chemical space.
8. **Unclear on how we determine desirable compounds and at what point are biological assays done?** All library compounds will be screened initially as their radioactive complex in the cell line expressing αvβ3 integrin receptor. Those that bind most tightly are the best. The most tightly bound that do not bind with the negative control cell line will be evaluated further.
9. **How will ELISA assay data be used an is selectivity or potency most important?** The proposed ELISA assay has been dropped and a new screening assay will be used. Initially potent compounds will be identified then only those potent compounds will be evaluated for selectivity.
10. **Will cell internalization be done will all compounds or a subset?** There are no internalization studies in our new bioassays.
11. **Milestone of two publications is peculiar and should be patent applications:** The reference to publications was made because the methods derived in this proposal will be of great research (not commercial) use in the medical arena utilizing chelating agents such as nuclear medicine, x-ray contrast imaging and MR imaging. Of course, CCTI will pursue any and all intellectual property rights that it can.
12. **Definition of "best" compounds ill defined and biological assessment needs more thoughtful evaluation of library members:** We have revised the biological assessment to be a series of tests wherein each subsequent test will have fewer members passing the criteria; first test is a general binding screen, then on to selectivity, then on to ability to functionally block the αvβ3 receptor in two cell lines.
13. **Desire to increase the binding efficiency by 10X and in vivo tumor localization by 2X seem modest:** We have dropped the animal models until phase II studies. The increase of 10X is a standard medicinal chemistry measurement that one can say is a meaningful increase in bioactivity achieved through lead optimization studies.
14. **Unclear if CCTI has a facility to conduct this research:** CCTI has its own modest laboratory facility to conduct the synthetic chemistry part of this proposal. The other collaborator's facilities will be used for the complexation, radiochemistry, and biological activity assessment.
15: **Committe budget recommendation to drop animal costs of $18,000:** This money request and animal experiments have been dropped and the money applied to brings Dr. Durden's expertise in integrins and cell signaling to bear on the project.

RESEARCH PLAN

A SPECIFIC AIMS

The proposed research has the following specific aims:

1) Develop and communicate new solid-phase synthetic methodology for macrocyclic chelating agents. MILESTONE: successful library production (>1000 members), at least 2 publications (and patent applicatons).

2) Preparation of $\alpha_v B_3$ integrin antagonists based around conformationally restricted chelating agents complexed with therapeutic radioactive metal ions. MILESTONE: biologically confirmed $\alpha_v B_3$ antagonist activity equal to or greater that of c(RGDfV).

3) Design and construct multivalent $\alpha_v B_3$ integrin receptor binding molecules possessing superior retention at the target site (tumor neovasculature). MILESTONE: successful synthesis of multivalent construct having 10X higher in vitro binding affinity.

This proposal represents an opportunity for experts in several disciplines (chelating agents, vascular biology, combinatorial chemistry, nuclear medicine, medicinal chemistry) to come together to capitalize on tumor vasculature targeting strategies to selectively deliver therapeutic radioisotopes to $\alpha_v B_3$ integrin-postitive tumors. This is to be accomplished using a novel and genreral approach mimicking antibody-type interaction via spatial arrangements of recognition units using conformationally restricted metal-ligand complexes as scaffolds.

B SIGNIFICANCE

Background and Existing Knowledge

Cancer research has been increasingly focused on tumor vasculature as a potential target for new therapies. Agents such as angiostatin and endostatin have been discovered which can potentially prevent the formation of new blood vessels (angiogenesis) and thus prevent further growth of solid tumors[1,2].

More recently another approach has been described which seeks to take advantage of the differences between normal tissue vasculature and the new vasculature (neovasculature) supporting tumors for the purposes of selectively targeting of drugs to tumors. These differences in vasculture have been noted in the physiology[3] of tumors as well as more recently at the molecular genetic level[4] of endothelium tissue. Monoclonal antibodies (Mabs) that recognize tumor vasculature specific antigens have been labeled with the alpha-emitter isotope ^{213}Bi and found to extend the life-span of tumor laden mice[5]. However, monoclonal antibodies as delivery agents in humans have significant hurdles in becoming therapeutic delivery agents[6]. In particular, Mabs, proteins and large polypeptides suffer from many problems as *in vivo* agents and, in fact, Bristol-Myers Squibb gave up work on angiostatin only last year in favor of developing small molecules that would mimic the effects of the large proteins[7].

Tremendous advances have been made in finding small molecules such as peptides that will target specific receptors *in vivo*. For example Erkii Rusolahti and Renata Pasqualini of the Cancer Research Center at Burnham Institute, La Jolla, Calif., have used phage display peptide libraries to find low molecular weight peptides containing the RGD (Arg-Gly-Asp) sequence that attach selectively to endothelial cells in the vasculature of tumors 40-80 times higher than to endothelial cells in other tissues[8]. The tumor associated receptors for these peptides appear to be the $\alpha_v\beta_3$ integrins which are receptors for vascular growth factors[9]. The $\alpha_v\beta_3$ receptor is widely reported to be highly expressed on many tumor cells (osteosarcomas, neuoroblastomas, glioblastomas, melanomas, and carcinomas--lung, breast, prostate, and bladder)[25]. The number of receptors per cell, an important consideration in targeting therapies where quantities of drug delivered are important, has been estimated to be up to 125,000 per expressing endothelial cell[25]. However, it should be noted that while $\alpha_v\beta_3$ integrin is selectively expressed in angiogenic blood vessels versus normal endothelial cells there are other sites *in vivo* that also express this receptor under normal conditions (notably osteoclasts[26]). The RGD-containing peptide sequences isolated by Rusolahti, possesing high binding selectivity for the $\alpha_v\beta_3$ integrin receptor have been tagged with anticancer drugs such as doxorubicin[8,10] and shown to enhance the efficacy of the drug against human breast cancer xenografts in nude mice versus the unmodified doxorubicin control. This was the first example of using the selective localization of a low molecular weight ligand binding to tumor vasculature-associated $\alpha_v\beta_3$ integrin to deliver a therapeutic anticancer drug.

The use of the peptide approach to bind with $\alpha_v\beta_3$ integrin receptors exploiting radionuclides as the toxiphore, targeting the neovasculature of tumors, has been proposed[11] but only limited work has been published[19,20]. The most detailed study examined several radioiodinated cyclic RGD peptides which were modeled after the previously optimized cyclo-(-Arg-Gly-Asp-D-Phe-Val-) pentapeptide system. For this cyclo-pentapeptide series they found that a hydrophobic amino acid in position 4 (D-Phe substitution) increases the receptor affinity whereas the position 5 (valine substitution) had little influence on the affinity. This series of cyclo-pentapeptides (including the iodinated tyrosine replacement for D-Phe analog called P2) were shown to be nanomolar inhibitors of the vitronectin receptor $\alpha_v\beta_3$ integrin. Moreover, they were selective for the $\alpha_v\beta_3$ integrin receptor over the $\alpha_{IIb}\beta_3$ receptor which is a glycoprotein involved in platelet aggregation. In order to avoid side effects that would be anticipated by affecting the platelet aggregation process it is critical that the affinity for the widespread $\alpha_{IIb}\beta_3$ integrin receptor is very minimal. Thus, all studies on $\alpha_v\beta_3$ integrin binding need to include a comparison binding study with $\alpha_{IIb}\beta_3$ integrin to evaluate this important parameter. The biodistribution data of the analog radioiodinated $\alpha_v\beta_3$ integrin binding peptide P2 is shown in the Table 1 below. Good initial localization in the tumors is noted but very quick clearance over a short 4 hour time period occurs[19]. The blood component clears even more quickly resulting in increasing tumor/blood ratios from 10 minutes to one hour time but essentially remaining constant through the four hour time period. The thyroid accumulates considerable isotope which is probably due to *in vivo* deiodination. Lastly, there is significant liver localization early on diminishing over time consistent with hepatobiliary clearance of the peptide. The loss of activity from the tumor site is not discussed by the authors but could be due to the lack of internalization of the antagonist at the receptor site. These results indicate that from a therapeutic standpoint there remains some optimization to be performed on this cyclo-pentapeptide system.

Table 1. Evaluation of radioiodinated tyrosine-containing cyclo-pentapeptide P2 [cyclo-(-Arg-Gly-Asp-D-Tyr-Val-)] in mice bearing tumors19 shown as % Injected Dose/gram

Tissue	Melanoma M21			Osteosarcoma			Mammary Carcinoma		
	10 min	60 min	240 min	10 min	60 min	240 min	10 min	60 min	240 min
Tumor	2.07	1.30	0.41	3.50	1.46	0.92	1.84	0.74	0.72
Blood	0.77	0.17	0.06	1.72	0.17	0.12	0.73	0.10	0.09
Muscle	0.42	0.25	0.10	0.94	0.36	0.24	0.48	0.16	0.14
Liver	21.96	11.23	0.78	19.06	4.22	2.18	25	12	1.33
Thyroid	2.21	3.45	0.3	3.49	15.61	30.02	5.40	1.88	4.90
Tumor/Blood Ratio	2.7	7.7	6.8	2.0	8.6	7.7	2.5	7.4	8.0

Habner and coworkers have extended the use of this cyclic pentapeptide, as described in recent presentations, by attaching the radioisotopes F-18, ^{188}Re, ^{90}Y and ^{99m}Tc to closely related derivatives of c(RGDfV) wherein the V (valine) has been replace by K (lysine) covalently modified on the epsilon-amino group[23,24] to contain a moiety capable of binding the radioisotope. The published data[23,24] showed a similar pattern of diminished absolute amount of isotope located at the tumor over time after initial uptake but accompanied by increasing tumor-to-blood ratios. This is the same pattern noted in Table 1 indicating that the loss of tumor associated activity over time is not due to the inherent biological clearance problems associated with iodinated biomolecules but must be due to a pharmacokinetic process.

The appeal of employing a radionuclide in this approach, targeting neovasculartue of tumors, is that no drug has to be liberated to perform the therapy and the radiation could be effective in either destroying the tumor-supplying blood vessels or directly destroying the tumor cells themselves since the site of the neovasculature localization is in such intimate proximity to the tumor cells in small metastatic lesions. Ideally, the radiation selectively localized to the neovasculature of metastatic tumors could work via both of these mechanisms if the proper radioisotope is utilized. For example, the penetration distance for the maximum energy particle (β^-)

emitted for $^{153}Sm^{+3}$ is estimated at only 3.4 mm versus 8.6 mm for $^{166}Ho^{+3}$. Thus, the choice of isotope should be matched to the phamacokinetics of the delivery agent as well as the size of tumor being treated. The potential value of just targeting the destruction of the neovasculature alone should not be underestimated as it has been estimated [11] that 100 tumor cells die for each destroyed endothelial cell in tumor blood vessels illustrating a possible amplification of the therapeutic localization of radioisotopes in tumor neovasculature.

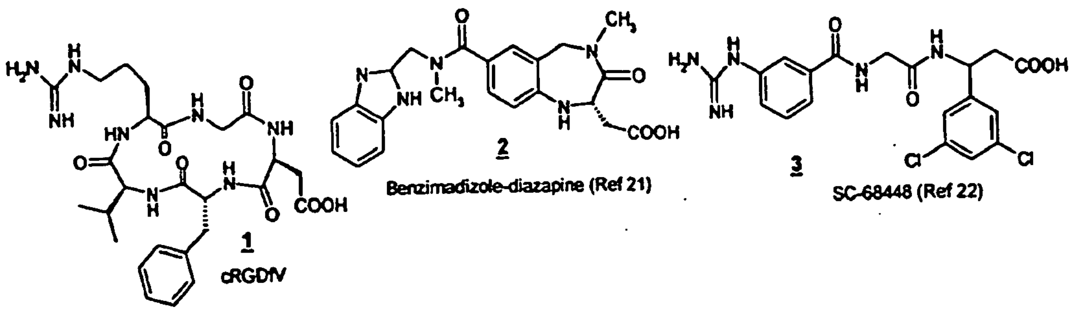
One drawback or disadvantage to using radioiodinated peptides such as the vascular targeting agents described above in Table 1 to selectively target tumors is their susceptibility to natural levels of peptidases and proteases which leads to extremely fast clearance rates from the bloodstream. While this may sometimes be useful for imaging purposes to yield a better target-to-nontarget ratio it is unacceptable in a therapeutic approach as it lowers the absolute amount of drug reaching the target[12]. Additional problems exist with radioiodinated peptides as opposed to chelated-metal-labeled peptides and that is the radioiodinated peptides are converted to iodotyrosines and iodide both of which clear quickly from the targeted site making the agent unacceptable in a therapeutic setting[12]. The obvious remedy of using a bifunctional chelating agent to attach radiometal ions to peptides, as an alternative to radioiodination, also presents problems in that because of the low molecular weight of the peptides (versus monoclonal antibodies) the presence of the attached metal complex can dramatically affect the biodistribution and pharmacokinetics of the low molecular weight radiolabeled peptide. In fact, a recent review stated that various studies have demonstrated "the essential role that the chelation and conjugation chemistries play in determining the *in vivo* uptake and phamacokinetic behavior of radiolabeled receptor-avid peptides being designed as potential therapeutic radiopharmaceuticals"[13]. Thus, **a peptide that has been optimized for targeting a receptor is likely to be suboptimized when a chelated metal ion is then conjugated to it.** This can be attributed to the addition of significant molecular weight as well as significant changes to the lipophilicity, molecular electronics, and steric environment of the ligand with regard to specific receptor binding interaction.

Investigators have studied the use of peptidomimetics to overcome the peptide limitations described above (fast clearance, metabolization) with some notable successes. For example, β-peptides have been used with success to mimic peptides as demonstrated by a cyclic β-tetrapeptide as a mimetic of somatostatin[14]. A more dramatic example is the use of nonpeptide-like templates used to present mimetics of individual key binding residues of peptides in their interactions with a receptor. The cyclic peptide bioactive somatostatin is represented in binding by a very different-looking mimetic based on β-D-glucose[15,16]. Binding assay results support the hypothesis that the glucose template (scaffold)-based presentation of binding groups can mimic somatostatin's biological activity.

This same approach did not work as well in the area of designing peptidomimetics for the $\alpha_v\beta_3$ antagonist cyclo(-Arg-Gly-Asp-D-Phe-Val-) [abbreviated as cRGDfV, 1] based on a carbohydrate template. In this work of Nicolaou et al. they first determined the solution structure of cRGDfV by NMR[17]. Based on molecular modeling Nicolaou proposed and synthesized a handful of cRGDfV analogs based on the pyranose carbohydrate ring system as a template. Unfortunately, little to no binding of these mimics to α_vB_3 integrin was observed. The authors suggest that there may exist subtle requirements for the active cyclic peptide conformation which may not be fulfilled by these mimics as well as perhaps a lack of sufficient rigidity associated with the carbohydrate framework[17].

Others have been more successful in finding peptidomimetics of cRGDfV (1) based on other templates. Benzodiazapines such as structure 2 have been found to be low-nanomolar inhibitors of vitronectin binding to α_vB_3 integrin with a 10000-fold selectivity over undesirable inhibition of $\alpha_{IIb}\beta_3$ receptor[21]. In this case the 1,4-benzodiazepine acts as a Gly-Asp mimic with the benzimidazole unit acting as an arginine mimic. Another RGD peptidomimetic selective inhibitor of α_vB_3 integrin was identified[3] (3, SC-68448) which showed up to 80% reduction in tumor growth in a mouse-based Leydig cell tumor model[22]. This molecule is simply an open chain analog presenting a guanidine moiety (arginine mimic) and a carboxylic acid (aspartic acid mimic) separated by a spacer group which allow for their presentation in a spatial arrangement that recognizes the α_vB_3 integrin

Figure 1. Structure of c(RGDfV) and nonpeptide mimetics.



1 cRGDfV

2 Benzimadizole-diazapine (Ref 21)

3 SC-68448 (Ref 22)

cyclo-(-Arg-Gly-Asp-D-Phe-Val-)

receptor. It should be noted that 2 and 3 are not disclosed as targeting agents but are examples of cRGDfV peptidomimetics that are selective α_vB_3 integrin receptor antagonists (selective relative to the $\alpha_{IIb}\beta_3$ receptor).

Commercial Opportunities

ComChem Technologies Inc. (CCTI) is a start-up company formed to discover and commercialize diagnostic and therapeutic radiopharmaceuticals. CCTI's strategy is to utilize combinatorial chemistry in conjunction with chelating agent expertise to explore new areas and to arrive at commercializable products quicker than its competition. This requires close collaboration with others possessing complementary expertise such as radiochemistry, medicine, and biochemistry.

CCTI has a competitive advantage in that the PI of this research proposal has a proven track record in inventing, developing, and bringing therapeutic radiopharmaceuticals into human clincal trials. He was instrumental in the development and first human trials of FDA approved Quadramet (licensed by Dow to Cytogen) as well as lead inventor and project champion for all aspects of ^{166}Ho-DOTMP which has now progressed to phase III human clinical trials (STR licensed by Dow to NeoRx Corporation).

The technology that will be developed in this proposal has a specific commercial application but also has broad application as a new method to produce three-dimensional presentation of molecular recognition units in a compact molecular space that is ideal for radiotherapy. The intellectual property expected to be generated herein will be protected by filing US and overseas patent applications.

Importance of Proposed Research

This Phase I work will lay the foundation for preclinical and clinical evaluation of tumor vasculature localizing radiotherapy for cancer treatment in Phase II. This agent will be broadly applicable to treating all α_vB_3 integrin-positive solid tumors with targeted radiotherapy. It has taken over 15 years for a monoclonal antibody (Rituxan) to finally achieve FDA approval for treating lymphoma. A radiolabeled version recently finished phase III trials and has been submitted to the FDA for approval. We believe the use of combinatorial chemistry applied to the problem of finding an optimum radiolabeled low molecular weight vascular localizing agent will allow for much faster discovery and development timelines. The commercial potential of this approach is enormous and the cost-of-goods expected to be much lower than an antibody approach which should result in a lower cost of the drug from the patient's perspective.

C **RELEVANT EXPERIENCE. Principal Investigator;** Dr. Garlich, CCTI Chief Scientist, has eleven years of industrial experience at Dow Chemical in the area of radiopharmaceutical discovery and development. He was instumental in the synthesis and formulation development for ^{153}Sm-EDTMP, an FDA approved radioactive drug for the relief of bone pain associated with bone metastases, licensed to Cytogen Corp.(Quadramet™). He also developed new azamacrocycles (synthesis and new uses) as well as bifunctional chelating agents for monoclonal antibodies. He is the father of ^{166}HoDOTMP, a bone-seeking radiopharmaceutical, now in phase III clinical trials for the treatment of multiple myeloma (licensed by Dow to NeoRX). More recently, he was responsible for establishing the combinatorial chemistry group at Dow

AgroSciences and has experience in all aspects of combinatorial chemistry-automation, solid-phase and solution phase synthesis, analytical instruments and methodology.

Co-Investigator; Professor Mark A. Green (Purdue University) has a background in inorganic chemistry and 18 years of productive research experience in the design, synthesis, and evaluation of new metal-based radiopharmaceuticals. His group is internationally recognized for their efforts in development and pre-clinical testing of low-molecular-weight copper radiopharmaceuticals for imaging with positron emission tomography. For tumor imaging, his group has also pioneered efforts in tumor targeting with low molecular weight folate-chelate conjugates that target a tumor-cell-membrane-associated receptor for folic acid. In addition, they have developed and evaluated an extensive series of monocationic gallium radiopharmaceuticals that are substrates for transport by the MDR1 P-glycoprotein involved in tumor multidrug resistance.

Project Coordinator; Carla J. Mathias (Purdue University) brings a background in zoology and chemistry to this project, along with 21 years experience in the design, synthesis, pre-clinical testing, and clinical evaluation of new radiopharmaceuticals. She is experienced in techniques of radiochemical synthesis and analysis, as well as the development and application of animal models for assessment of new radiopharmaceuticals. Her experience includes synthetic, animal, and human studies related to the evaluation of radiolabeled platelets and white cells, radiolabeled antibodies, 18F-labeled estrogen receptor ligands for imaging breast tumors with PET, generator-based PET perfusion tracers, and low molecular weight radiopharmaceuticals targeted to tumor-associated receptor systems.

Co-Investigator: Dr. Don Durden, M.D., Ph.D. (Indiana University Medical School), has 5 years experience and expertise in vascular biology and the study of aniogenesis and integrin signaling. He has hands-on experience with the avB3 integrin receptor and signaling. He is an expert in the biochemical and molecular dissection of signal transduction pathways in mammalian cells. In addition to being a board certified Pediatrician and Pediatric Oncologist he will actively participate in the development and running of meaningful biological assessment studies of the compounds produced from this grant.

D RESEARCH PLAN:

Experimental Plan Stage A & B Rationale and Introduction

Given the drawbacks and approaches described above in the Background section it would be desirable to treat cancers that are highly expressing $\alpha_v B_3$ integrin by a small nonpeptide molecule that 1) possesses a built-in chelating agent complexed with a therapeutic radioactive metal ion in a stable fashion and 2) the resulting nonpeptide metal-ligand molecule possesses a high affinity and selectivity to the $\alpha_v B_3$ integrin. We propose to achieve this with conservation of atoms by using the chelating agent moiety itself as the template upon which to place the $\alpha_v B_3$ integrin binding moieties in a spatial arrangement that mimics the well known $\alpha_v B_3$ integrin antagonist c(RGDfV). The synthesis involved in this approach is detailed in Stage A below. Expanding on this approach is our proposed design to use the chelating agent as the platform from which to tether multiple copies of a selective $\alpha_v B_3$ integrin-binding moiety such as c(RGDfV). This multivalent approach (Stage B), a relatively new concept and not yet applied to integrin binders, will be approached combinatorially to find the optimum distances between the multiple copies of the binding moiety and to study the effect of different spacing groups on the binding of the resulting construct with integrins. The astute reader will recognize after examining the generic schemes that there is some crossover from Stage B into Stage A in that some of the members of Stage A can contain multiple copies of presented binding moieties. This is not an intent to confuse the reader but reflects the great flexibility built into the synthetic approaches.

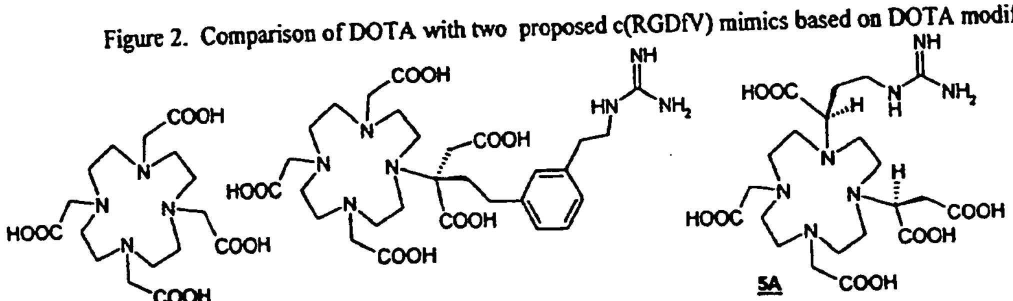
Synthesized molecules that mimic the binding of monoclonal antibodies are called chemobodies[35]. We have coined the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding. Compounds described in both Stage A and Stage B fit into this new category of chelabodies.

Research Plan Stage A: Preparation of RGD Mimics Based Upon Macrocyclic Complexes (Chelabodies)

The chelating agent DOTA, 4 (1,4,7,-10-tetraazacyclododecane-tetraacetic acid), is well know to form kinetically inert complexes with the lanthanides[28] and the resulting complexes are considered conformationally ridgid[29]. The resulting complexes are overall negatively charged at physiological pH when complexed with a trivalent metal ion. The attractiveness of a complex utilizing lanthanides as the metal ion is attributable to the variety of radioactive lanthanides in use in nuclear medicine ($^{153}Sm^{+3}$, $^{90}Y^{+3}$, $^{166}Ho^{+3}$) with differing half-lives and beta-particle energies. The lanthanides tend to be quite similar in their complexation chemistry so that the design of one system may allow the use of any one of several therapeutic radioactive lanthanide metal ions (ie thus more flexibility in choosing the proper radioisotope based upon biological half-life). It should be noted that the Principal Investigator has extensive experience (synthesis, complexation, and radiochemistry expertise) with lanthanides and macrocyclic chelating systems that has led to one commercial drug (Quadramet) and one drug in Phase III clincal trials (STR being evaluated by NeoRx Corporation). Another attractive feature of the DOTA chelator system is its widespread use in clinical MRI imaging agents and bifunctional chelating agents for attaching radioactive lanthanides to monoclonal antibodies for use in humans.

An inspection of molecular models of DOTA complexes indicates that DOTA is similar in size to the peptide ring $\alpha_v B_3$ integrin antagonist c(RGDfV). This led us to the idea that suitable c(RGDfV) mimics could be prepared by judicious subsititution patterns on the DOTA backbone. For example, molecular modeling indicates that structure 5 (DOTA-RXG)when complexed with Y^{+3} would place the guanidine and carboxylic acid in a similar spatial arrangement as that found for the guanidine of the arginine and the carboxylate of the aspartic acid residues in c(RGDfV)[29]. Likewise, from modeling estimates structure 5A (upon complexation with Y^{+3}) appears to also satisfy the spatial requirements of the binding moieties of c(RGDfV)[29]. Stucture 5 represents a single arm attachment and structure 5A represents adjacent chelating arm modifications. It should be noted that modeling indicates that similar achievement of a c(RGDfV mimic using modifications of acetate arms that are not adjacent would be difficult unless extremely large and conformationally floppy spacer groups are used. Thus our effort will be focused initially on 5 and 5A and their analogs.

Figure 2. Comparison of DOTA with two proposed c(RGDfV) mimics based on DOTA modifications.



DOTA

4

DOTA-RXG

5

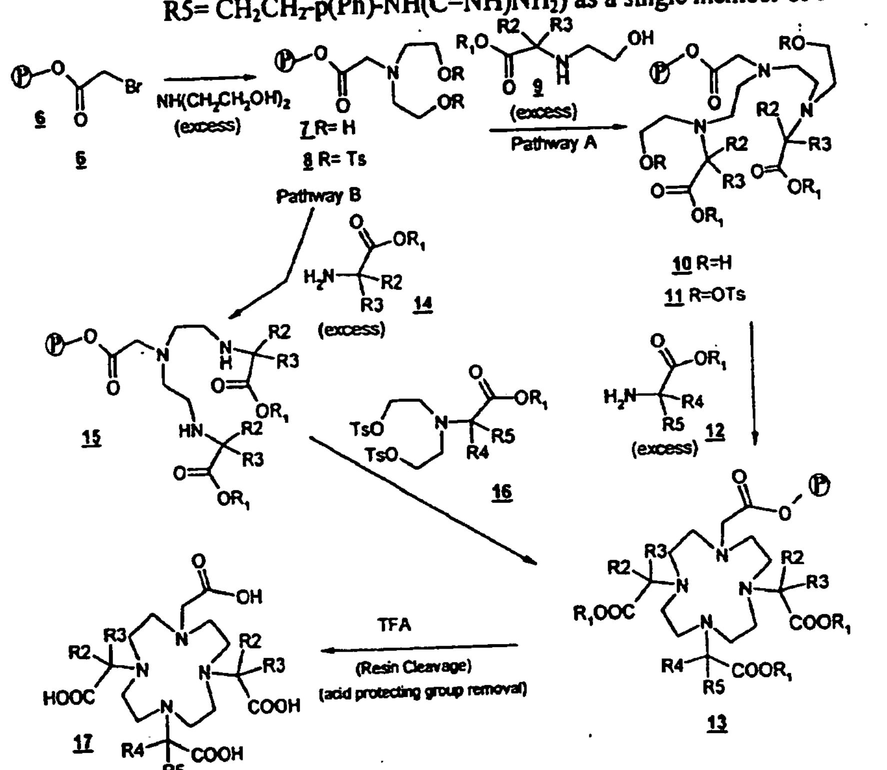
5A

There are numerous other possible substitutions on the acetate arm besides those shown in 5 and 5A which could restrict rotation even further to provide additional preorganization to mimic c(RGDfV). Additionally there are many additional groups that can serve as carboxylate mimics and guanidine mimics. Our plan is to prepare a library of compounds similar to 5, guided by molecular modeling, via the solid-phase combinatorial chemistry route proposed in Figure 3.

In Figure 3 the circled P represents the solid phase resin, Wang resin in this case. However, the use of Rink amide resin is also to be evaluated which would give a DOTA-based chelator wherein one of the chelating acetate arms is a -CH2C(O)NH2 group upon cleavage from the resin. These types of chelators are known and while they are not as stable as DOTA they are stable enough for *in vivo* use[29]. An additional advantage of this monoamide from Rink amide resin would be that the resulting complex with trivalent lanthanides would give a neutral complex core molecule. This could have important *in vivo* biodistribution effects which will be studied.

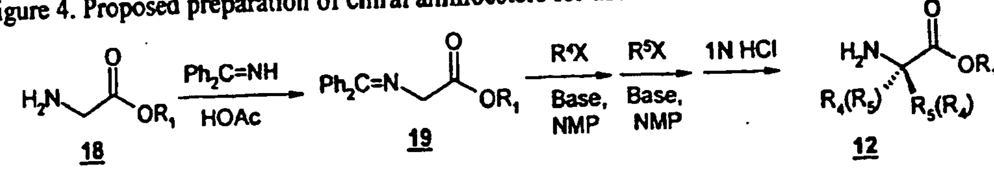
* The synthetic scheme (Figure 3) to prepare these molecules illustrates two pathways to get to the same desired substituted DOTA chelator, 17. Both pathways will be examined and each will require significant optimization work. These efforts would represent the first on-resin synthesis of the medically important tetraazacyclododecane ring system. We thus feel that this work, even if ultimately unsuccessful in the biological evaluation, will be a welcome and exciting combinatorial chemistry methodology advance in the area of chelation based inorganic medicinal chemistry. By using R2=R3=H the synthesis as shown in Figure 3 simplifies to only one chelator arm substituted with two moieties. The stereochemistry is not shown in Figure 3 but the use of the proper enantiomer of 12, which we plan to isolate and obtain in each instance, will deliver the desired stereoisomer as shown in structure 5.

Figure 3. Proposed solid-phase synthesis of 5 (R2=R3=H; R4=CH_2COOH; R5= CH_2CH_2-p(Ph)-NH(C=NH)NH_2) as a single member of a combinatorial library.



* The key building unit to get to structures like 5 via the route shown in Figure 3 is a chiral unnatural amino acid derivative. A diverse collection of these disubstituted glycine derivatives can be prepared in solution phase or solid phase by the UPS (unnatural peptide synthesis) route pioneered by O'Donnell who is serving as a consultant on this proposal[31,32]. This procedure is shown in Figure 4 and lends itself to automation[33]. It is anticipated that the different enantiomers resulting in Figure 4 will be separated using chiral chromatography. There are methods to perform the chemistry in Figure 4 wherein either R4 or R5 is hydrogen with significant stereoselectivity (80-90% ee) but our criteria for purity (>95%) requires that we perform a chiral separation at this stage. This will be performed using HPLC methodology.

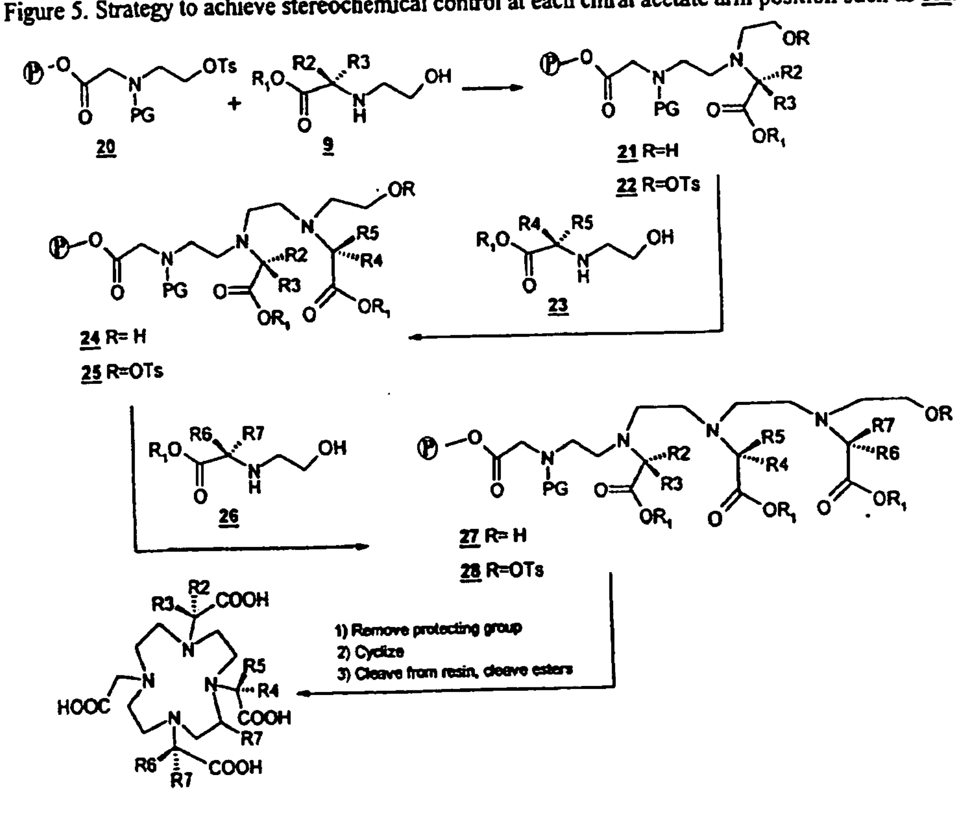
Figure 4. Proposed preparation of chiral aminoesters for use in combinatorial synthesis(Figure 3).



With the inputs 12 (and 14 which can be the same or different from 12, derived from the same chemistry) in hand then the library production protocol based on structure 5 can be developed. Because of the way the synthesis is developed it is possible to make an analog of 5 where each of the three acetate arms contain one copy of the RGD mimic structure by making 12 and 14 the same aminoester. This trivalent species, by benefit of compact presentation of three copies of the RGD mimic strucure, could possess some interesting properties. There is more discussion later regarding this multivalent approach in the research plan stage 2 discussions.

In order to access desired target molecules such as 5A a different synthesis route is needed since two identical molecules of aminoester are incorporated in either pathway A or pathway B in Figure 3. This uncontrollable dual incorporation precludes introducing the needed stereochemistry at both sites, i.e. only one acetate substitution pattern will have the correct configuration. To address the desired access to molecules like 5A and to give complete control over the stereochemistry of all 6 substituents on the chelating acetate arms the synthetic protocol shown in Figure 5 will be evaluated. The amino alcohols 9, 23, and 26 will be prepared from the corresponding unnatural amino esters prepared by the method shown in Figure 2 and purified to get the single isomer. The preparation of these aminoalcohols could make use of resin bound ethylene glycol wherein the amine of the amino ester (such as 12) displaces the activated non-resin bound hydroxyl of the ethylene glycol. The PG (protecting group) on the nitrogen of Figure 5 will be determined after some preliminary work is

Figure 5. Strategy to achieve stereochemical control at each chiral acetate arm position such as 5A.



performed to ensure othogonal stability but likely will be a group such as FMOC, NOSYL, or trifluoracetamide.

The reviewers at the last submission of this grant indicated they wanted a better feel for what types of compounds are going to made in these combinatorial libraries. It should be noted that although we plan to do combinatorial synthesis we will do so in a fashion such that each well has one intended compound and not intentionally prepare mistures of compounds which tends to confound bioassay interpretation. The best way I know to give you a feel for the types of compounds we would be making is to throw out a chart of representative monomers which would

be put together in all possible combinations (i.e. combinatorially) to generate a library for screening. This approach is shown in Figure 5A. Structure 5 is reproduced as one of the targets that molecular modeling indicates has a reasonable chance of being an RGD mimic. Rather than believe that molecular modeling is the final answer we assume that molecular modeling gets us into the ball park and we will use combichem to get us the optimize compound. Thus, structure 5 can be thought of as a specific example of the generic structure illustrated by 35, where L is a covalent linkage, BA is a basic amine capable of accepting a proton, and AG is an acidic group capable of donating a proton and being negatively charged. The use of bioisosteres in medicinal chemistry is well documented wherein substitutions are made which resemble the original group or moiety (for a good review see "Bioisosterism: A Rational Approach in Drug Design, Chem. Rev. 1996, 96, 3147). This is for example how the stuctures 2 and 3 were discovered (Figure 1) and found to be nonpeptide mimetics of C(RGDfV) as discussed in the background material. With this concept in mind and switching to a combinatorial mode we can pick a number of BA (basic amine groups) that would mimic the guanidine moiety with a single attachment mode for connectivity to L. A sample of these are shown in Figure 5B where the dashed line represents the covalent attachment to the rest of the molecule. These substutuents can vary from simple amines (53,) of which a large number are available commercially and can be chosen to maximize diversity (using medicinal chemistry principles or computational methods), or can be heterocyclic in nature or various substituted guanidines.

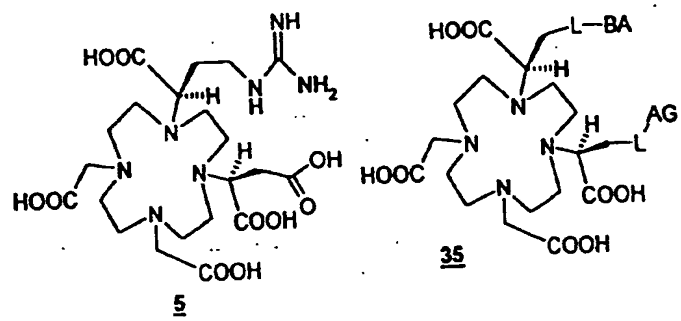
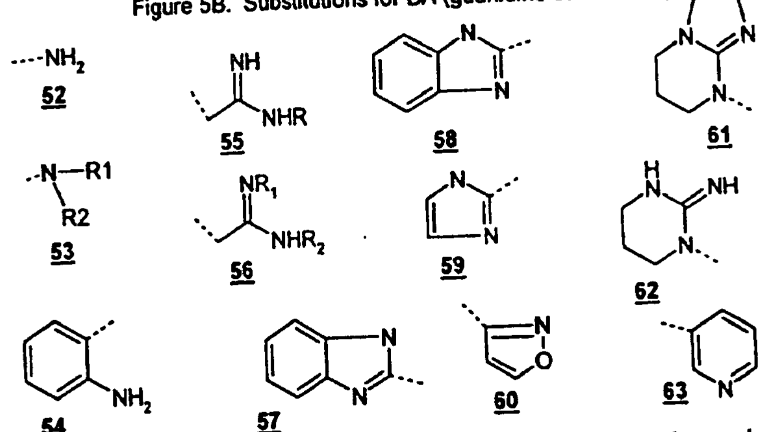


Figure 5A. Generic form of desired target 5

Figure 5B. Substitutions for BA (guanidine substitution)

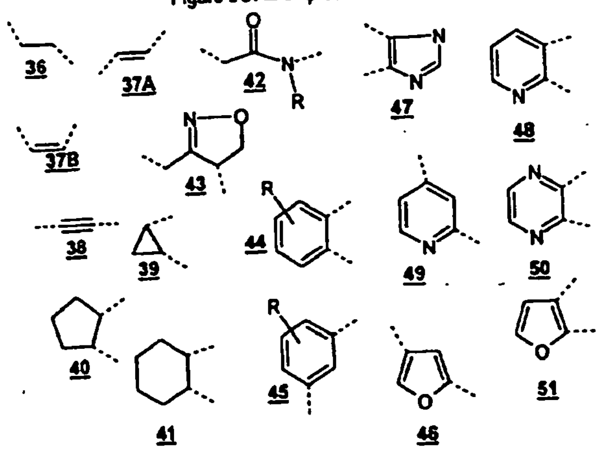


Likewise for the connection (linker) L a number of resonable linkages can be prepared which would serve to spatially separate the receptor binding group (AG or BA) in the proper orientation and distance. Examples of potential L groups are shown in Figure 5C. The dashed lines in the structures represent the points of connectivity. The reader realizes that in most cases (unsymmetrical) the site of attachment can be reversed to give a different linkage. The actual attachment chemistry varies for each one; for example most are envisioned as carbon-carbon bonds but some can be more synthetically accessible if they are heteroatom attachment (ie ethers).

* Lastly, the carboxyl group of c(RGDfV) or of 5 can be conceptually replaced with any of a number of isosters to carboxylic acids. A partial list is shown in Figure 5D ranging from the obvious sulfonic acid (67) or phosphonic (68) to the tetrazole 71. Moreover, with this group there are also substitution wild cards as shown by the "R" groups in Figure 5D. These then represent potentially hundreds of compounds that could be utilized in putting this collection together.

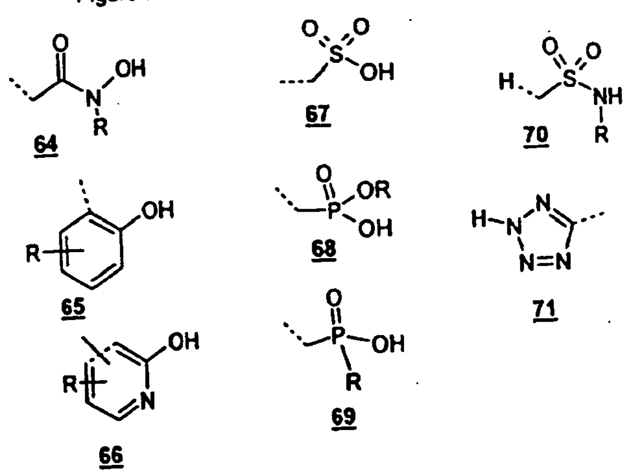
Just looking at the few monomers illustrated here we can see a 12X17X8 (guanidine substitutions X linkers X carboxylic acid analogs) proposed library (1632 individual compounds) built just around the structure 5 hypothesized by molecular modeling to be a reasonable fit for RGD cyclic peptide.

Figure 5C. Examples of linkers L



These proposed chelator scaffolds (chelabodies) addresses all of the shortcomings described previously for a tumor neovasculature seeking agent. The positive attributes for this system are 1) nonpeptide in nature so not prone to metabolism; 2) incorporates a kinetically inert lanthanide complex which allows for a potential range of radioisotopes having varied particle energies and half-lives and yet produced commercially (Sm-153, Ho-166, and Lu-177); 3) rigid backbone (cyclododecane ring system locked into place upon chelation) upon which to place appropriately spaced recognition/binding groups; 4) the complex containing the toxiphore (radioactive metal ion) is part of the core rigidifying structure so no additional conjugation chemistry is required, i.e. the compound from screening will not need to be further modified to label with a radioactive isotope;

Figure 5D. Possible Groups for AG (acidic carboxyl group)



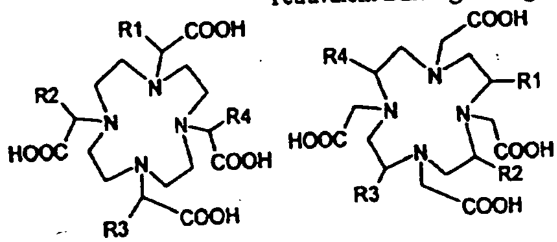
Research Plan Stage B: Preparation of Extended Multivalent RGD Mimics Based Upon Macrocyclic Complexes (Chelabodies)

Monoclonal antibodies are known for their exquisite selectivity and high binding affinity. These attributes arise in part because antibodies are divalent and in some cases multivalent in their binding with proteins or receptor surfaces. Nature has used multivalent binding to overcome weak binder in order to make strong attachments[35]. Multivalency, simultaneous attachment of two or more binding sites on one molecule (drug) to multiple receptor sites on another (cell surface), is a new approach to drug design according to George M. Whitesides of Harvard University[35,36]. This multivalent approach has not yet been applied to ligands aimed at binding the integrins although Burgess has disclosed a cyclic sequence, c(RGDRGD), that could be considered a dimer of RGD[37]. Surprisingly this ligand possessed excellent selectivity and antagonistic activity towards $\alpha_v B_3$ integrin.

This area of multivalent drug design is where the term "chemobody" has been coined to describe synthesized molecules that mimic the binding of monoclonal antibodies[35]. We are proposing the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding.

Research plan stage B comprises the design and evaluation of multivalent presentations of α_vB_3 integrin antagonists based on the DOTA template. This is illustrated conceptually in Figure 6 where either four substitutions are made on the chelating arms (30) or situated around the macrocyclic ring (31). We have also considered the possibility of a mixed species where some substitution is on the acetate arms and some is on the backbone carbons but no compelling reason exists to pursue this approach over the other two described here in more detail. Given the resource available in this proposal we will put our effort in the arm substituted system (30) since that approach takes advantage of the chemistry worked out in research plan A. The focus of this proposal is for the R groups to contain, preferably at their terminus, a moiety that is an α_vB_3 integrin antagonist. The ideal terminal group would be one that induces internalization of the bound ligand into the cell and compounds will be tested for this property (see biological assay section). In order to prove the concept involved here we first will use known antagonists at the terminal binding positions. For example the known antagonist c(RGDfK) (32) has been described and is amenable to capping off the "R" arms to provide a suitable multivalent antagonist construct. This compound will either be synthesized in-house or custom prepared for CCTI outside of the budget requested here. The linker/spacer arms can be similar to those described in the literature for multivalent constructs, some of which are illustrated in Figure 7. One basic linker arms idea is to react carboxylic anhydrides with a nucleophile such as nitrogen on the arm stub and then couple a diamine with the resulting free carboxylic acid. This procedure is amenable to solid-phase synthesis to prepare arms that are all the same[38,39]. Applying this strategy to the compounds of Figure 4 and Figure 5 requires only that some of the substituents (R2, R3, R4, R5, R6, R7) on the arm building blocks (9, 12, 14, 16, 23, 26) contain a masked electrophile (to react with amines for example) or nucleophile (to couple with carboxylic acids for example) that can be deprotected and then elaborated into a linke/spacer module for endcapping with antagonists such as 32. This approach would work via the chemistry outlined in Figures 4 and 5 to give essentially trivalent constructs (i.e. one per each substituted chelator arm). There is no convenient method to get to a fully symmetrical tetravalent system using solid phase methodology so solution phase methods will be examined. It is apparent that there are a large number of possible constructs that could be prepared varying the nature and length of the arms.

Figure 6. Conceptual design of Chelabodies Based on DOTA-type Chelating Agents Presenting a Tetravalent Binding Arrangement Aimed at α_vB_3 Integrin Antagonism.



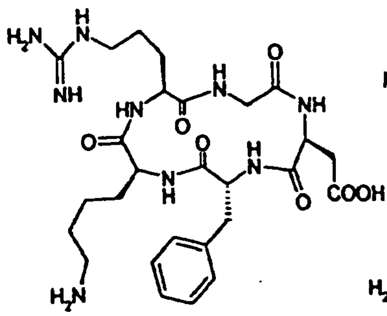
30 31

R1,R2,R3,R4= Z-M

Z= linker/spacer of varible length, shape, flexibility

M= RDG mimic that selectively antagonizes avb3 intetrin

Figure 7. Proposed Endcap Moiety for α_vB_3 Integrin Antagonist in a Multivalent Construct and Examples of Linker/spacer Modules.



32

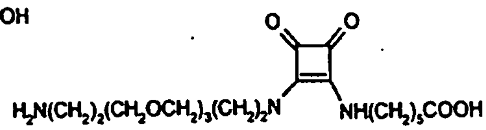
c(RDGfK)

cyclo-(-Arg-Gly-Asp-D-Phe-Lys-)

$NH_2CH_2CHCH_2\text{-}(OCH_2CH_2)_3\text{-}CH_2NH\text{-}COCH_2CH_2COOH$

(ref 38)

33



(ref 39)

34

Our approach is to prepare a combinatorial library of such constructs and to assess their biological binding and performance (*in vitro* binding and whole cell assays) to determine if improvements in tumor cell localization are possible.

Research Plan Biological Evaluations:

Overall Summary: The purpose of this aspect of the proposal is to identify and characterize scaffolding chelate molecules which bind specifically to the αvβ3 integrin. Once candidate compounds are identified, assays will be designed to confirm RGD specific binding, αvβ3 or αvβ5 specificity and determine binding affinities for each molecular construct. Finally we will examine physiological consequences of RGD chelate action on integrin functions and whether the RGD chelate is internalized into the cell. All of these effects will be carefully compared with RGD, RGDfV, LM609 (anti-αvβ3) and P1F6 (anti-αvβ5) effects on αvβ3 and αvβ5 functions (adhesion, migration and internalization of integrins).

Assessment of Biological Activity of Library members and lead compounds: In the original proposal we proposed assaying via the *in vito* ELISA test common in the literature for identifying avb3 antagonists. The reviewers felt that we did not have enough expertise for this because the avb3 integrin receptor is a finicky bioassay to run. In this revision we have pulled in Dr. Don Durden who certainly has the expertise to ensure we can run those assays. However, recent developments (cited by the previous proposal's reviewers) indicate a more realistic testing can be performed with a melanoma cell line that expresses the avb3 integrin receptor on its surface[44,45]. We will initially use the M21 melanoma cell line to screen a large number of organic scaffolding chelate compounds for binding to avb3 (see references 46, 47 for background on these lines). The M21 human melanoma cell line (M21L) which is αvβ3 negative has been engineered to express αvβ3 (termed M21L4) will be supplied by our long term collaborators David A. Cheresh, Scripps Clinic Research Foundation and Peter C. Brooks, New York University [47].This cell line is αvβ5 negative. Once specific avb3 binding constructs are identified we will use the human and bovine brain derived microvascular endothelial cell lines (HBEC and BBEC), which have been carefully characterized in our laboratory, express αvβ3, αvβ5, α5β1 and α2β1. Importantly, these integrins will serve as controls for specificity of interaction of RGD mimetic compounds in a physiologically relevant cell type i.e. microvessel derived human endothelial cell. We will use monoclonal antibodies specific for each integrin and flow cytometry to confirm expression levels of each specific integrin subunit in these cell lines at regulate intervals (anti-αvβ3, LM609; anti-αvβ5, P1F6; anti-α5β1, P4C10; anti-α2β1, 19522) [48]. These monoclonal antibodies are function blocking in that they block association between the integrin and its natural ligand or RGD determinant e.g. avb3, vitronectin. The expression levels in our hands has been stable over several years of study. We will utilize these cells in conjunction with specific peptides and monoclonal antibodies to test for specific binding of RGDfV and RGD mimetic organic chelates for targeting to angiogenic endothelial cells. The effects of αvβ3 antagonism with LM609 antibody or cyclic RGD antagonism

have been studied extensively in the Durden laboratory in these cell lines and other endothelial cell lines[49]. We now apply this experience to evaluation of organic RGD mimetic chelates.

Briefly M21 cell variants, positive or negative for αvβ3 integrin, will be incubated in the presence of different concentrations of radiolabeled chelate constructs. The concentrations that we will start with will be experimentally determined with these cell lines by first conduting probe studies with the gold standard and well charactized c(RGDfV) peptide. Depending on the numbers of library members to evaluate we may pick a level 20X more concentrated than that found for c(RGDfV) binding as our cut-off point to find interesting leads. Binding assays will be performed in 96-well plates for high through-put screening. In saturation binding assays cells will be incubated with various concentrations of chelates diluted in binding buffer, containing 20mM Tris-HCL, pH 7.4, 100 mM NaCl and 2 mM $CaCl_2$). Cells will be exposed to the chelate for 60 minutes at 37 degrees C, in the presence or absence of RGDfV or RADfV cyclic peptide or competing nonradiolabeled cold chelate. After incubation, integrin associated chelate is separated from unbound by extensive washing of cells will ice cold binding buffer. Remaining cell bound chelate is quantitated by lysis of cells and liquid scintillation counting or gamma counting of whole cell lysate. In competition binding assays, various concentrations of nonradioactive chelate was added to a set of wells to determine specificity of the interaction of radiolabeled compounds. In these assays we will perform controls to examine the capacity of RGDfV and RADfV cyclic pentapeptides to block association of the chelate molecules with M21L4 cells. We will label these compounds by chelating them with radioactive lanthanides e.g. for pure β emission we will use Y-90 and for γ emission we will use Sm-153 or Ho-166 isotopes. Detection of these radiolabeled RGD chelate constructs will be performed using standard scintillation counting or a gamma counter. By and large the stability constant for each DOTA-based chelate for the metal ion should be unaffected by the RGD-mimicing moieties attached. All lead complexes passing the biological screens will be examined for complex stability in aqueous solution as a funtion of time (using an HPLC-MS based analytical assay).

We will screen a large number of candidate scaffolding chelate molecules to identify a relatively small number which bind to avb3. We will evaluate the capacity of these chelate constructs further for the capacity to functionally block the αvβ3 receptor. Several assays will be performed in both M21 and HBEC cells in this regard: 1) adhesion to different matrix proteins including fibronectin, vitronectin and type IV collagen 2) migration of HBEC and M21 cells in a haptotaxis assay using fibronectin and vitronectin matrix proteins. As a control, the capacity of M21 and HBEC cells to migrate on vitronectin will require expression of αvβ3 and be inhibited by the LM609 function blocking monoclonal antibody. The capacity to migrate on fibronectin or fibronectin peptides will not be affected by LM609 or P1F6 monoclonal antibodies and will not be affected by the RGD specific chelate molecule.

In this we way we can carefully evaluate the capacity of the RGD chelate molecules to bind to avb3/avb5 and block avb3/avb5 function under physiologic relevant conditions. The next phase of biological evaluation will be to examine binding affinity of the RGD chelate molecules in comparison to cyclic RGDfV or RADfV and/or the monoclonal antibody LM609 or vitronectin binding itself. For these assays we will employ scatchard analysis using radiolabeled RGD specific chelate constructs shown to bind in an RGD specific manner to avb3 or avb5 positive cells. Once specificity is established we will turn our attention to examination of affinity of binding to HBEC and M21 avb3 integrins using cold non-labeled RGD chelate molecules or in competition with agents known to bind to avb3 on these cells i.e. RGDfV or LM609 monoclonal antibody. It would be expected that RGDfv and not RADfv would displace the RGD chelate. It is not predicted that LM609 specific epitope of avb3 will be competed by the RGD chelate molecule. From these combined experiments we expect to determine which RGD chelate molecule binds to cellular avb3 in RGD dependent manner and determine binding specificity and affinity for each chelate found to associate with avb3. We will determine relative binding affinity of chelates as compared with RGDfV. Finally we expect to determine if function blocking activity is present in each RGD

chelate construct. Lastly we will determine if these RGD chelate molecules are internalized by M21 or HBEC cells following binding to the cell surface.

To confirm these results we will utilize biotinylated purified vitronectin, the $\alpha v\beta 3$/avb5 specific ligand, and examine the capacity of RGDfV, RADfV versus RGD chelate to block binding of biotinylated vitronectin to $\alpha v\beta 3$ on M21 or avb3/avb5 on HBEC cells. This analysis would be performed using flow cytometric analysis using an avidin conjugated to APC fluorescent dye for quantitation of vitronectin binding to the cell. We will also examine the capacity of the various RGD chelate molecules to alter binding of biotinylated or radiolabeled RGD peptide with avb3/avb5 on HBEC and/or M21 cells. Cold RGDfV and not RADfV will be expected to compete for binding to avb3. In parallel, we will evaluate the capacity of RGD chelates found to bind to avb3 to compete for RGD binding. A scatchard analysis of this interaction will determine the relative affinity of these RGD chelates for interaction with avb3/avb5 as compared to RGDfV or LM609 or P1F6 or vitronectin.

It would be expected that the capacity to block vitronectin binding would correlate with affinity of RGD chelate to avb3/avb5 (results obtained above). These results would serve to further verify specificity and binding affinity of specific RGD chelate constructs. The bound constructs can be then verified by mass spectroscopy by elution or HPLC analysis of cell lysates as compared with original synthesis material for each chelate molecule. This would confirm the molecular identity of the chelate selectively bound to the $\alpha v\beta 3$/$\alpha v\beta 5$ on the cell. An alternative approach will involve the exposure of avb3 positive cells (M21L4) to the chelate as a method for screening a library of combinatorial compounds to determine which species will bind by performing mass spectroscopy of cell lysates following exposure to a library chelate constructs and extensive washing steps. Controls will include M21 cells which do not express avb3 integrin and do not display RGDfV binding or antagonism of avb3 specific adhesion to vitronectin *in vitro*.

Alternatively we will purify $\alpha_{IIb}\beta_3$ or $\alpha v\beta 3$ integrin dimers from human platelets or placenta using a combined lectin and RGD affinity chromatography as described[50,51] and incorporate this integrin into liposomal particles for determination and screening for RGD chelates which bind to avb3 (see reference 52)). As a negative control we will purify a5b1 (fibronectin receptor) and α2b1 (collagen receptor) integrins as controls for these experiments. Importantly we will first attempt experiments in HBEC and M21 cells since this is more reflective of in vivo state of avb3 and a5b1. If the use of these cell lines is met with problems we will turn our attention to the purification of integrins approach (a more complete description of assays using isolated receptors was in the original submission of this proposal and is still present at the end of the biological assessment section but only as a back-up assay if needed).

It is recognized that the avb3, avb5 and a5b1 do not function in a vacuum and are highly linked in terms of physiologic function. It should be stated that the integrin heterodimer function is highly complex linked to a diverse network of intracellular signaling cascades. Integrins are involved in ligand induced changes in affinity and avidity which impact on the binding of integrins to specific ligands (matrix proteins) and to the transmission of intracellular signaling (inside-out versus outside-in signaling). The role of divalent cations in the RGD chelate-integrin interaction may require further experiments. Our (DLD) laboratory is actively involved in the study of complex network of signal transduction events which occur upon avb3 and a5b1 engagement. It is hard to bind to one integrin without affecting the physiologic function of another. In this regard, it is known that RGD and vitronectin bind to avb3 and avb5 integrins and that both are expressed in angiogenic sites and tumors. Hence an RGD chelate which binds to both avb3 and avb5 will be a possible outcome of these experiments and would be expected to have antitumor activity. Accordingly, experimental conditions for determining binding of RGD chelate molecules to RGD determinants in avb3 or avb5 may require further manipulation of experimental conditions to achieve our goal, which is to identify molecules that bind to αv integrins and to examine the effects of this binding on integrin function.

Back-up Literature based Assay-In Vitro : The ELISA-type in vitro testing for competitive binding of test ligands with $\alpha_v B_3$ integrin is well established as are the methods to obtain the needed starting materials; vitronectin, $\alpha_v B_3$ integrin, firbrinogen, and $\alpha_{IIb} B_3$ integrin[19, 22, 27, 41, 42, 43]. Briefly, the solid-phase competitive displacement *in vitro* assay test comprises; 1) coating 96-well plates with $\alpha_v B_3$ integrin receptor (or $\alpha_{IIb} B_3$ integrin receptor to determine selectivity), 2) washing sequence including 1% BSA, 3) exposure to various concentrations of test compound containing biotinylated vitronectin (or biotinylated fibronectin)[19] for 2 hours, 4) washing sequence, and finally 5) detection of biotin present using reporter-labeled anti-biotin antibody. This testing will be performed on nonradioactive metal ion complexed with our newly synthesized compounds so that it can be performed in a medium-throughput mode at the Purdue Center for Combinatorial Chemical Biology.

Specific Goals/Accomplishments Expected for Phase I Year 1:

1 Perform modeling of complexes (chelabodies) that will mimic neovasculature targeting peptide-receptor binding interactions via substitution patterns on a DOTA-lanthanide complex scaffold.
2 Several virtual libraries of complexes are assessed by molecular modeling of receptor fit to determine synthetic direction have been performed.
3 Synthetic methodology has been developed to create macrocyclic chelator based libraries that are mimics for the c(RGDfV) binding ligand.
4 Biological assessment screening protocols are developed to screen libraries, some libraries have been evaluated and some hits are identified..
5 Hits from biological screens are confirmed, identified and synthetic effort to optimize at least some of these hits has been initiated including follow-up focused libraries.
6 Work has begun to evaluate the feasibility of making multivalent constructs. Some constructs will have been prepared.

Specific Goals/Accomplishments Expected for Phase I Year 2:

1 Leads from research plan stage A have been optimized and have been fully characterized *in vitro* and are ready for preclincial studies.
2 Synthetic methodology has been developed for preparing multivalent constructs in research plan stage B.
3 Multivalent construct libraries from research plan stage B have been prepared and hits optimized from *in vitro* bioassays to give the most potent compounds.

Brief Glimpse into Phase II STTR:
In vivo evaluation of the best *in vitro* active compounds will be the initial activity. The animal testing we will perform will follow those most recently published in the area of nuclear medicine[19]. These animal results using human tumors implanted into immune-compromised mice will provide biolocalization data. We will not be measuring antitumor effects as the animals will be sacrificed to quantitate the tumor and normal tissue uptake. The use of animal pharmacokinetics will be valuable information to go back and make additional compounds based on what we learn *in vivo* (ie, too hydrophobic, too hydrophilic, clears too fast, to slow, sticks in kidney, etc.). In phase II we envision quick iterations of animal studies, resynthesis/remodeling, biotesting and then back to animals. The goal of phase II is to maximize tumor localization in several animal models and minimize nontarget localized radioactivity. Some efficacy studies in tumor bearing animals is envisioned also for phase II.

E HUMAN SUBJECTS- NONE

F VERTEBRATE ANIMAL-NONE

G CONSULTANTS-NONE

H CONTRACTUAL ARRANGEMENTS- Upon receiving funding for this STTR proposal the collaboration between CCTI and Dr. Mark Green of Purdue University to accomplish this research proposal will be formalized with a research contract. Likewise, the inclusion of Dr. Durden in the bioassay part of this collaboration (a major revision from first submission) will also be formalized with a research contract. Support letter to this effect are attached. Dr. Durden's inclusion allows the project to access his considerable expertise in vascular biology including $\alpha_v B_3$ integrin signal transductions and angiogenesis.

I LITERATURE CITED

1. Fan, T.P., Jaggar R., and Bicknell, R. Trends Pharmacol Sci 1995, 16 (2), 57.
2. Folkman, J. Nature Med. 1995, 27.
3. Brown, J.M., and Giaccia, J.A. Cancer Research 1998. 58, 1408.
4. Croix, B. St., Rago, C., Velculescu, V., Traverso, G., Romans, K.E., Mongomery, E., Lal, L., Riggins, G.J., Lengauer, C., Vogelstein, B., Kinzler, K.W. Science 2000, 289, 1197.
5. Kennel, S.J. and Mirzadeh, S. Nuclear Medicine & Biology 1998, 25, 241.
6. Dickman, S. Science 1998, 280, 1196.
7. Barinaga M, Science 1999, 283, 1831.
8. Arap, W., Pasqualini, R., Ruoslahti, E. Science 1998, 279, 377.
9. Pasqualini R, Kolvunen, E. , Ruoslahti, E. Nature Biotechnology 1997, 15, 542.
10. Arap, W. Pasqualini, R. Ruoslahti E., Current Opinion in Oncology 1998, 10, 560.
11. Pasqualini, R. Quarterly Journal of Nuclear Medicine 1999, 43, 159.
12. Blok, D. Feitsma, R., Vermeij, P., Pauwels, E.J.K. Eur J. Nucl. Med. 1999, 26, 1511.
13. Volkert, W.A., Hoffman, T.J. Chemical Reviews 1999, 99,2269 and references therein.
14. Seebach, D. and Hoyer, D. Angew. Chem. Int. Ed. 1999, 38, 1223.
15. Hirschmann, R., Nicalaou, K.C., et al. J. Am. Chem. Soc. 1993, 115, 12550.
16. Hirschmann, R., Nicalaou, K.C., et al. J. Am. Chem. Soc. 1992, 114, 9217.
17. Nicolaou, K.C., Trujille, J.I., and Chibale, K. Tetrahedron 1997, 53, 8751.
18. Peczuh, M.W. and Hamilton, A.D. Chem. Rev. 2000, 100, 2479.
19. Haubner, R. et al., J. Nucl. Med. 1999, 40, 1061.
20. Line, B.R., et al. J. Nucl. Med. 1999, 40, 79P, abstract number 319.
21. Keenan, R.M. et al. J. Med. Chem. 1997, 40, 2289.
22. Carron, C.P. et al. Cancer Research 1998, 58, 1930.
23. Haubner, R. et al. J. Nucl. Med. 2000, 41, 42P, abstract number 162.
24. Bock, M. et al. J. Nucl. Med. 2000, 41, 41P, abstract number 160.
25. See references in reference 19.
26. Personal communication, Renata Pasqualini, November 14, 2000.
27. Wong, A. Hwang, S.M., et al. Mol. Pharmacol. 1996, 50, 529.
28. Volkert, W.A., Hoffman, T.J. Chemical Reviews 1999, 99, 2269.
29. Caravan, P. Ellison, J.J. et al. Chemcical Reviews 1999, 99, 2293.
30. Molecular modeling was kindly provided by Prof. Lipkowitz and Dr. G. Lombardo both of IUPUI Chemistry Department using the X-ray crystal structure of Y-DOTA complex and applying the universal force field program to arrive at an energy minimized structure (see J.Am. Chem. Soc. 1992, 114, 10024).
31. O'Donnell, M. J., et al. J. Am. Chem. Soc. 1996, 118,6070.
32. Scott, W.L., et al. Tetrahedron Lett. 1997, 38, 3695.
33. Griffith, D.L., et al. Tetrahedron Lett., 1997, 38, 8821.
34. O'Donnell, M.J. et al. Tetrahedron 1999, 55, 6347.
35. Chemical & Engineering News, 2000, October 9, page 52.
36. Mammen, M. et al. Angew. Chem., Int. Ed. 1998, 37, 2754.
37. Burgess, K., and Lim, D., J. Med. Chem. 1996, 39. 4520.
38. Rose, K. J. Am. Chem. Soc. 1999, 121, 7034.
39. Fan, E. et al. J. Am. Chem. Soc. 2000, 122, 2663.
40. Kitov, P. I. et al. Nature, 2000, 403, 669.
41. Smith, J. W., and Cheresh, D.A. J. Biol. Chem. 1988, 263, 18726.
42. Mitjans, F. et al. J. Cell Sci. 1995, 108, 2825.

43. Pytela, R. et al. Science 1986, 231, 1559.
44. Haubner, R. et al. Cancer Research, 2001, 61, 1781.
45. Haubner, R. et al. J. Nucl. Med. 2001, 42(2), 326.
46. Stromblad, S., Becker, J. C., Yebra, M., Brooks, P. C., and Cheresh, D. A, J Clin Invest. *98:* 426-33, 1996.
47. Petitclerc, E., Stromblad, S., von Schalscha, T. L., Mitjans, F., Piulats, J., Montgomery, A. M., Cheresh, D. A., and Brooks, P. C., Cancer Res. *59:* 2724-30, 1999.
48. Petitclerc, E., Boutaud, A., Prestayko, A., Xu, J., Sado, Y., Ninomiya, Y., Sarras, M. P., Jr., Hudson, B. G., and Brooks, P. C, J Biol Chem. *275:* 8051-61, 2000.
49. Erdreich-Epstein, A., Shimada, H., Groshen, S., Liu, M., Metelitsa, L. S., Kim, K. S., Stins, M. F., Seeger, R. C., and Durden, D. L., Cancer Res. *60:* 712-21, 2000.
50. Wong, A., Hwang, S. M., McDevitt, P., McNulty, D., Stadel, J. M., and Johanson, K., Mol Pharmacol. *50:* 529-37., 1996.
51. Wong, A., et al., J Pharmacol Exp Ther. *285:* 228-35., 1998.
52. Parise, L. V. and Phillips, D. R., J Biol Chem. *260:* 10698-707., 1985

[redacted]

Joseph R. Garlich, Ph.D.
ComChem Technologies, Inc.
9731 Trilobi Drive
Indianapolis, IN 46236

SCHOOL OF MEDICINE

RE: Chelate Based Scaffolds in Tumor Targeting (STTR FLAIR Grant)

Dear Joe:

I am writing to confirm that I am interested and willing to be a hands-on collaborator with you and Dr. Mark Green of Purdue in your efforts to target novel chelate-based radiopharmaceutical to the integrin vasculature supporting new tumor growth.

In Year one ([redacted]) I am able to devote 8% of my time for a salary cost of $10,942, fringe benefits of $1,880, chemical/biochemicals/assay supplies of $1,272 for a total of $14,094. Indirect costs (49%) add another $6,906 for a total in year 1 of $21,000.

In Year two [redacted] I am able to devote 7% of my time for a salary cost of $9,574, fringe benefits of $1,645, chemical/biochemicals/assay supplies of $862 for a total of $12,081. Indirect costs (49%) add another $5,920 for a total in year 2 of $18,001.

I look forward to contributing my expertise in vascular biology, angiogenesis, and integrin signaling to helping develop the biological activity assessment associated with this project.

Sincerely,

Donald L. Durden, M.D., Ph.D.
Associate Professor Pediatrics.
Biochemistry and Molecular Biology
Indiana University School of Medicine
Indianapolis, IN 46202

HERMAN B WELLS CENTER
FOR PEDIATRIC RESEARCH

James Whitcomb Riley
Hospital for Children
Indiana University
Medical Center
Cancer Research Institute
1044 W. Walnut Street
Room 402
Indianapolis, Indiana
46202-5225

317-274-8900
Fax 317-274-8679

PURDUE UNIVERSITY

SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

[redacted]

Joseph R. Garlich, Ph.D.
President
ComChem Technologies, Inc.
9731 Triboli Drive
Indianapolis, Indiana 46236

RE: Chelate-Based Scaffolds in Tumor Targeting

Dear Joe:

I am writing to confirm that my group is most interested in collaborating in ComChem's efforts to develop novel targeted chelate-based radiopharmaceuticals *via* application of combinatorial chemical techniques. Carla Mathias and I will be delighted to assist in your efforts to develop and evaluate radiopharmaceuticals targeted to tumor vasculature, as we have discussed and outlined in the accompanying subcontract proposal.

We look forward to working with you and Dr. Durden on this most exciting initiative.

Best regards,

Mark A. Green

Mark A. Green, Ph.D.
Professor of Medicinal Chemistry

MAG/ksk

DIVISION OF NUCLEAR PHARMACY • DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
1333 HEINE PHARMACY BUILDING • WEST LAFAYETTE, IN 47907-1333
(765) 94-1441 • FAX: (765) 494-1414

PURDUE UNIVERSITY

SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

[redacted]

Center for Scientific Review
National Institute of Health
6701 Rockledge Drive
Bethesda, Maryland 20892

RE: National Institutes of Health Application entitled, "*Chelate Based Scaffolds (Chelabody) in Tumor Targeting*" (J.R. Garlich, Ph.D., Principal Investigator, ComChem Technologies, Inc.)

To Whom It May Concern:

The appropriate programmatic and administrative personnel of each organization involved in the above-referenced application are aware of the PHS consortium grant policy and are prepared to establish the necessary inter-institutional agreements consistent with that policy. We understand that the grantee institution has the specific responsibility for ensuring that all required assurances are obtained

Sincerely,

Mark A. Green, Ph.D.
Professor of Medicinal Chemistry

Diane Troyer
Assistant Director
Sponsored Program Services

DIVI ION OF NUCLEAR PHARMACY • DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
1333 HEINE PHARMACY BUILDING • WEST LAFAYETTE, IN 47907-1333
(765) 494-1441 • FAX: (765) 494-1414

STATEMENT OF INTENT TO ESTABLISH A CONSORTIUM AGREEMENT

Date: [redacted]

Grant Number: n/a

Application Title: Chelate-based scaffolds (Chelabody) in tumor targeting

Proposed Project Period: [redacted]

"The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the NIH consortium grants policy and will establish the necessary inter-institutional agreement(s) consistent with that policy.

"Further, (1) the prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency, and (2) where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal."

ComChem Technologies, Inc.
Applicant Institution

Principal Investigator

Official Authorized to Sign for Institution

Indiana University
Consortium Institution

Principal Investigator

Official Authorized to Sign for Institution
Mark L. Brenner, Ph.D.
Vice Chancellor for Research and
and Graduate Education

Checklist

TYPE OF APPLICATION *(Check appropriate box(es).)*

☐ NEW application. *(This application is being submitted to the Public Health Service for the first time.)*

☒ REVISION of previously-submitted application number [redacted]
(This application replaces a prior unfunded version of a new application.)

☐ CHANGE of Principal Investigator *(if applicable)*
Name of former Principal Investigator ______________________

1. ASSURANCES/CERTIFICATIONS

The assurances/certifications set forth below are made and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (small business concern) on the FACE PAGE of the application. Descriptions of individual assurances/certifications are found in application instructions under "Checklist." If unable to certify compliance with any item, provide an explanation and place it after this page.

• Human Subjects; • Vertebrate Animals; • Debarment and Suspension; • Drug-Free Workplace; • Delinquent Federal Debt; • Research Misconduct; • Civil Rights (Form HHS 690); • Handicapped Individuals (Form HHS 690); • Age Discrimination (Form HHS 690).

2. PROGRAM INCOME *(See discussion in application instructions under "Checklist.")*

All applications must indicate *(Yes or No)* whether program income is anticipated during the period for which grant support is requested.

☒ No ☐ Yes *(If "Yes," use the format below to reflect the amount and source(s) of anticipated program income.)*

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COSTS *(See discussion in application instructions under "Checklist.")*

Insert the rate, if known. If the applicant organization does not have a currently negotiated rate with the Department of Health and Human Services (DHHS) or another Federal agency, it must estimate the amount of indirect costs allocable (applicable) to the proposed Phase I project. That amount should be inserted in the space provided below. The applicant organization should also be prepared to furnish financial documentation to support the estimated amount, if requested by the Public Health Service. An applicant organization may elect to waive indirect costs if it so desires.

☐ DHHS agreement, dated: ______________ : __________ % salary and wages or __________ % Total Direct Costs.

☐ No DHHS agreement, but rate established with ______________________________, dated: ____________

☐ Rate negotiation pending with the National Institutes of Health.

☐ Indirect costs allocable (applicable) to this Phase I project are estimated to be $ ______________

☒ No indirect costs requested.

4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workplace and/or promote the non-use of tobacco products or have plans to do so?

☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*

[redacted]

OIPE JC82
[redacted]
PATENT & TRADEMARK OFFICE

DISCLOSURE DOCUMENT NO.
468887
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

Document Disclosure Program
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

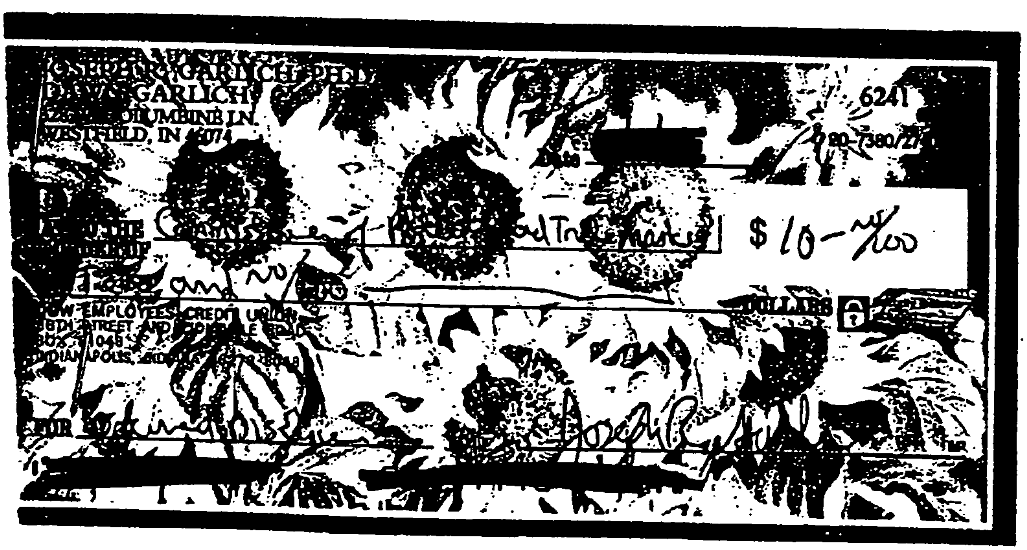
The undersigned, being the inventor of the disclosed invention, 3 pages, requests that the enclosed papers be accepted under the Disclosure document Program, and that they be preserved for a period of two years.

Enclosed is a Check for $10.00 to cover this submission. Thank you for your help.

Sincerely,

Joseph R Garlich

Joseph R. Garlich, Ph.D.
328 West Columbine Lane
Westfield, IN 46074

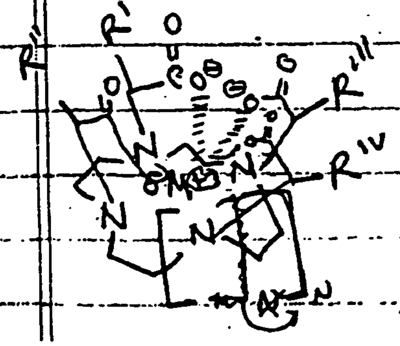


MACROCYCLIC MIMOTOPES USEFUL FOR (Therapy) DRUG TARGETING OR IMAGING IN MEDICINE

by: JOE GARLICH. Joseph R Garlich [redacted]

Mimetopes are peptides that mimic the structure of a folded protein. The spatial orientation of molecular binding interactions are ~~sure~~ crucial to molecular recognition events such as antibody recognition of a substrate, protein recognition of substrate. It is important for the proper spatial orientation to be a low energy form or otherwise preffered orientation. I propose using macrocyclic chelating agents which when complexed with appropriate imaging or therapeutic metal ions will form a conformationally restricted spatially oriented presentation of molecular recognition units (such as peptides, H-bond donors/acceptors, charge-charge interaction, lipophilic-lipophilic, hydrophilic-hydrophilic, pi bond stacking, Van der Waals interaction etc) that are useful in medicine for example binding selectively to membranes of cancer cells or other target cells or proteins. Some specifics are below:

1A) Macrocycles such as 1,4,7,10-tetraazacyclododecane are good for this purpose as the chelating "arms" (ie Nitrogen nonring substituents) are all situated on one "side" of molecule & held (locked) into conformation thru bonding with metal ion: (Non-limiting example)



where R', R'', R''', R^{IV} are independently chosen molecular recognition units or elements as illustrated above that together when complexed with a metal ion form spatial collection that recognizes & binds preferentially to some target protein or cell membrane

N = 0, 1 preferably to give cyclononane-triaza ~~cycle~~ dodecane-tetraaza

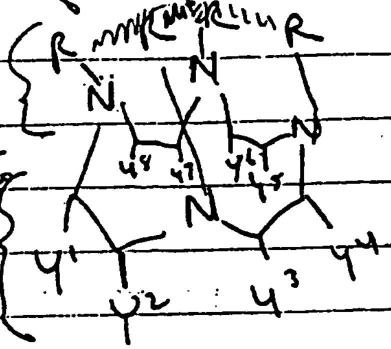
Joseph R. Garlich [redacted]

MACROCYCLIC MIMOTOPES; CONTINUED (J. GARLICH)

1b) Same as 1A except substituents are off of ethylene backbone of azamacrocycle Ring

Chelating polar side molecules

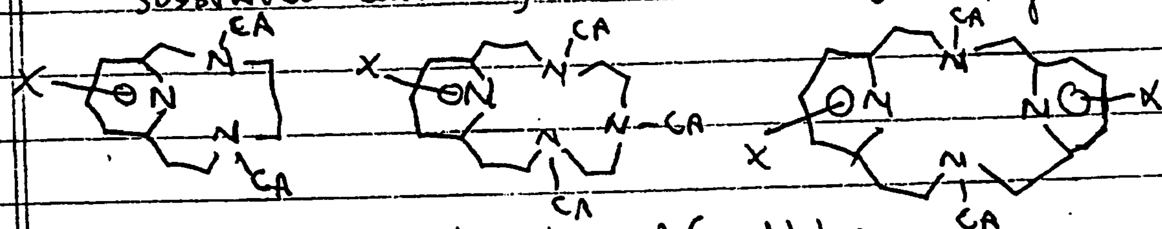
Backside substituting of ring backbone ethylene to give proper spatial arrangement of 1 or more Y groups for recognition purposes.



R = chelating groups

Y = independently chosen molecular recognition unit or elements as in 1A.

1C) SAME AS 1B, 1A except using a macrocycle base ring substituted containing 1 or more heterocycle rings:



where X = molecular recognition element (in addition to those present attached to "CA" or on ring (ie like Y groups in 1B & R groups in 1A) and "CA" = chelating arm - electron donor to metal

1D) Metals in 1A, B, C can be any mono, di, tri valent metal ions that are useful in imaging or therapy in medicine. Non-limiting examples are Fe, Sm, La, Ho, Ga, In, Tc, Re, Rh in various valences/charges & also including radio active isotopes thereof.

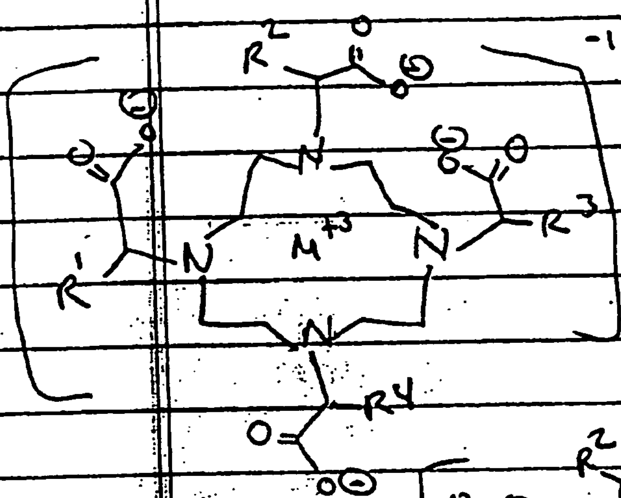
1E) 1A, 1B, 1C, 1D above where the chelating arms consist of electron donors from phosphonate, phosphonate halfesters & phosphinic moieties ie $-\overset{O}{\underset{}{P}}-O^-$; $-\overset{O}{P}{<}^{O^-}_{OR,Y}$, $-\overset{O}{P}{<}^{O^-}_{R,Y}$ where R, Y are molecular recognition elements

1F) 1A, B, C, D, E above where molecule (without metal ion) is synthesized using combinatorial chemistry with chelating arm attached to resin & elaborated using unnatural peptide synthesis methodology.

(Joe Garrecht) Joseph R Garlich [redacted]

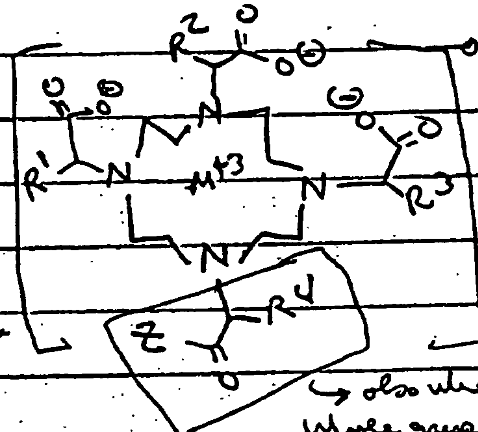
2) NONLIMITING EXAMPLES of 1A–1F:

$M^{+3} = {}^{153}Sm^{+3}$ or ${}^{166}Ho^{+3}$



R1, R2, R3, R4 are all independently selected to optimize binding target cell, protein, or molecule when chelator is complexed with metal ion to give proper spatial orientation of arm substituents

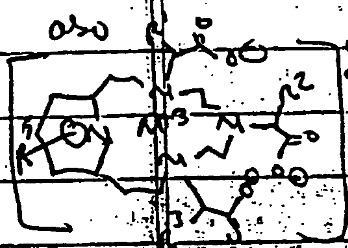
3) more of (2) (neutral complexes)



— o charge

same as 2 except acid group on one arm is not charged and is ester or amide group to give overall neutral core complex

also



— o charge

→ also where whose group is not glycine based, i.e. single N–R4 or N–Z type to give overall neutral complex

4) 2 & 3 above where one or more chelating arms are

$-CH(R)P(O)(O^-)O^-$; $-CH(R^a)P(O)(O^-)OR^b$; $-CH_2P(O)(O^-)OR$; $-CH_2P(O)(O^-)R$; $-CH(R^a)P(O)(O^-)R^b$

where R groups (including R^a, R^b) are independently selected molecular recognition elements

Joseph Garlich
328 W Columbine Ln
Westfield, IN 46074-9739

DISCLOSURE DOCUMENT NO.

470081

RETAINED FOR 2 YEARS

THIS IS NOT A PATENT APPLICATION

OIPE
FEB 2 8 2000
PATENT & TRADEMARK OFFICE

Approved for use through 6/...
Patent and Trademark Office; U.S. DEPA...
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays ...

PTO-1652 (8/99)

Disclosure Document Deposit Request

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: Synthesis of Novel Chelating Agents Using Solid Phase Synthesis Techniques

Enclosed is a disclosure of the above-titled invention consisting of 4 sheets of description and 0 sheets of drawings. A check or money order in the amount of 310.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

Joseph R. Garlich
Typed or printed name

328 West Columbine Lane
Address

Westfield

IN 46074
City, State, Zip

NOTICE TO INVENTORS

...stood that a Disclosure Document is not a patent application, nor will its receipt date in any way ... date of a later filed patent application. A Disclosure Document may be relied upon only as evidence ... on and a patent application should be diligently filed if patent protection is desired.

...ll be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be ... is referred to in a related patent application filed within the two-year period. The Disclosure Document may be ... transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired ... isclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

...d should not be considered to be a "grace period" during which the inventor can wait to file his/her patent ... oss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring ... ust usually also establish diligence in completing the invention or in filing the patent application since the filing ...

...at is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or ... about patent matters, you are advised to consult with an attorney or agent registered to practice before the ... Patent and Trademark Office, is available ... eys and Agents Registered to Practice Before the United States Patent and Trademark Office ... of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone ... Also, many large cities have associations of patent attorneys which may be consulted.

...ny public use or sale in the United States or publication of your invention anywhere in the world more than one ... ent application on that invention will prohibit the granting of a patent on it.

...ich have been understood and witnessed by persons and/or notarized are other examples of evidence which may ... iority.

...rk of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related ... e to the public, including automated access to PTO databases. Publications such as General Information Con... ... find out the location of the PTDL closest to ... als at the PTDLs, as well as the PTO's Web site at www.uspto.gov. ... the PTO's Web site or in every issue of the Official Gazette, or call the ... omplete listing of all PTDLs that appears on the PTO's Web site or in every issue ... (703-308-4357). To ensure assistance from a PTDL ... Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP ... to contact a PTDL prior to visiting to learn about its collections, services, and hours.

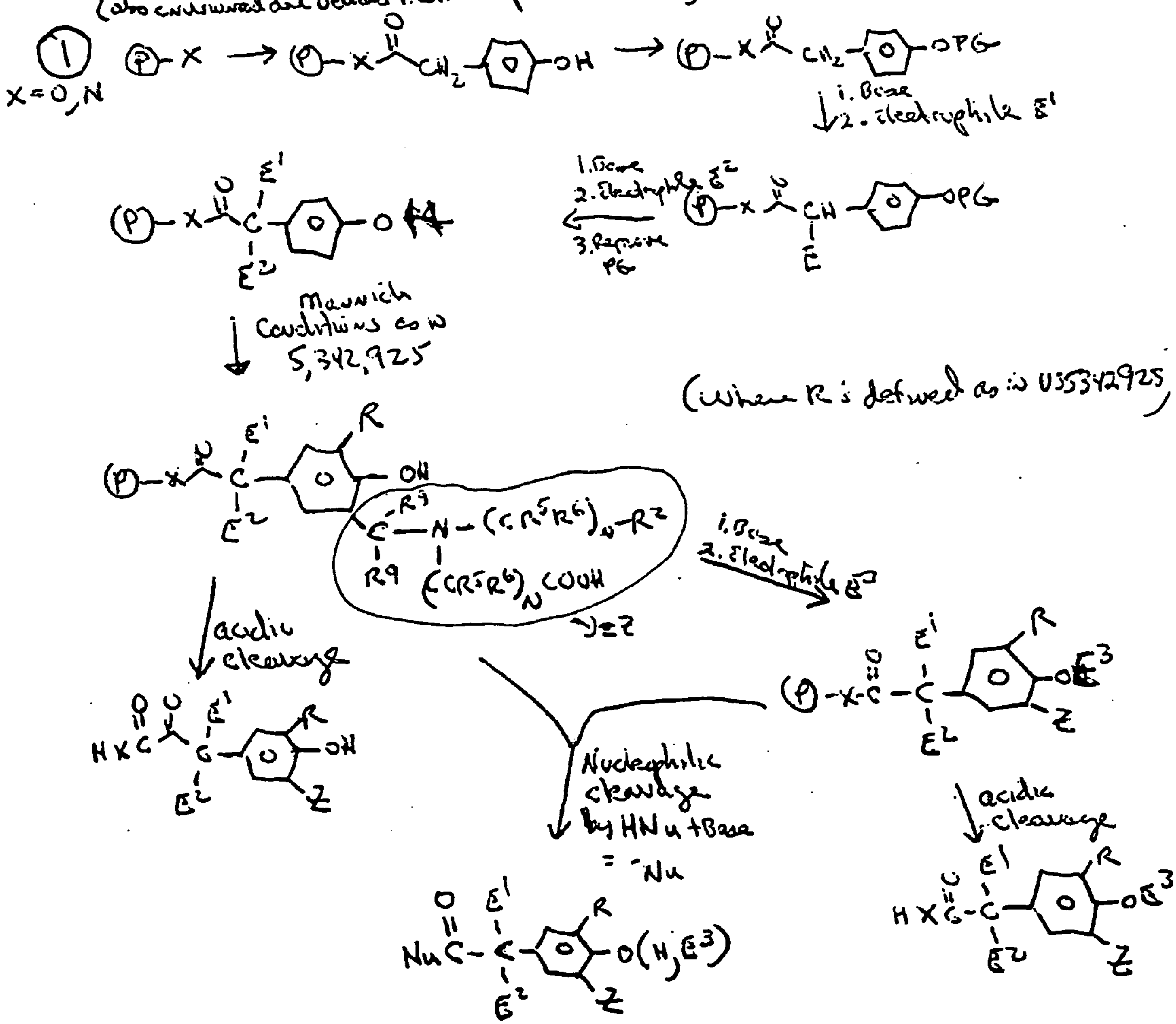
...collection of information is used by the public to file (and by the PTO to process) Disclosure Document Deposit Requests. ... USC 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, ... Disclosure Document Deposit Request to the PTO. Time will vary depending upon the individual case. Any comments on ... to complete this form and/or suggestions for reducing this burden, should be sent ... the Chief Information Officer, U.S. ... U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ... Commissioner for Patents, Washington, D.C. 20231.

[redacted] 8194

IDEAS ON SYNTHESIS OF NOVEL CHELATING AGENTS USING SOLID PHASE SYNTHESIS TECHNIQUES: By Joe Garlen Joe Garlen

US Patent 5,342,925 describes a limited set of chelating agents that were found to deliver metal ions to tumor for diagnostic or human therapeutic uses. All the compounds described therein were all made using traditional solution phase syntheses. I propose for medicinal purposes the compounds described below and the solid-phase synthetic routes to get to them: (P) represent a solid phase synthesis resin; PG=Protecting Group

(also envisioned are various metal complexes thereof)



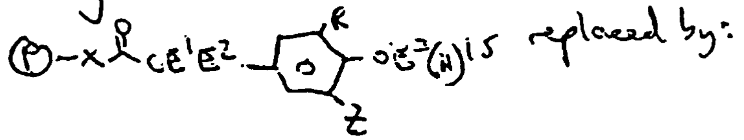
cont'd on next page

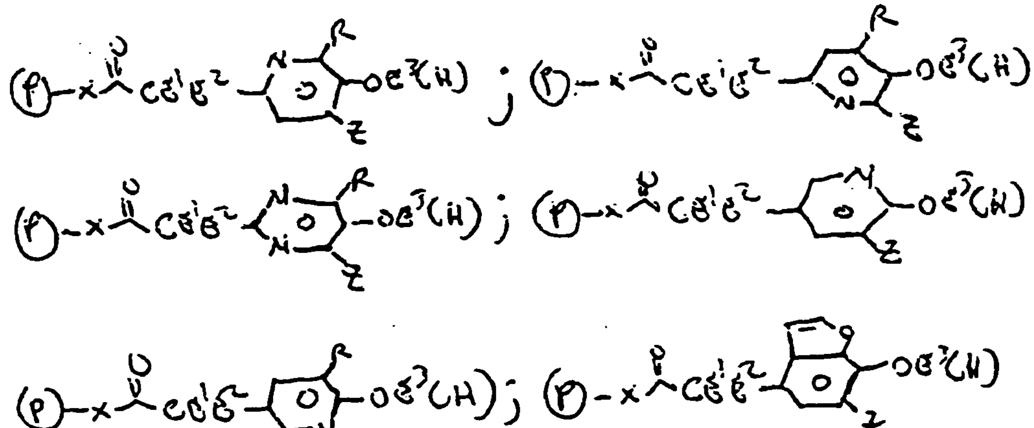
IDEAS ON SYNTHESIS OF NOVEL CHELATOR'S (CON'T)

(2) #1 above & 3 when X = N and is initialy a rink amide resin where one of the hydrogens on Nitrogen is alkylated via alkyl halide or tosylate or via reductive amination

ie $Ⓟ-X = Ⓟ-NH_2 \rightarrow Ⓟ-N(H)(R) \equiv Ⓟ-X$ w 1 above

(3) #1 above where the aromatic ring is a heteroaromatic ring such as but not limited to below:

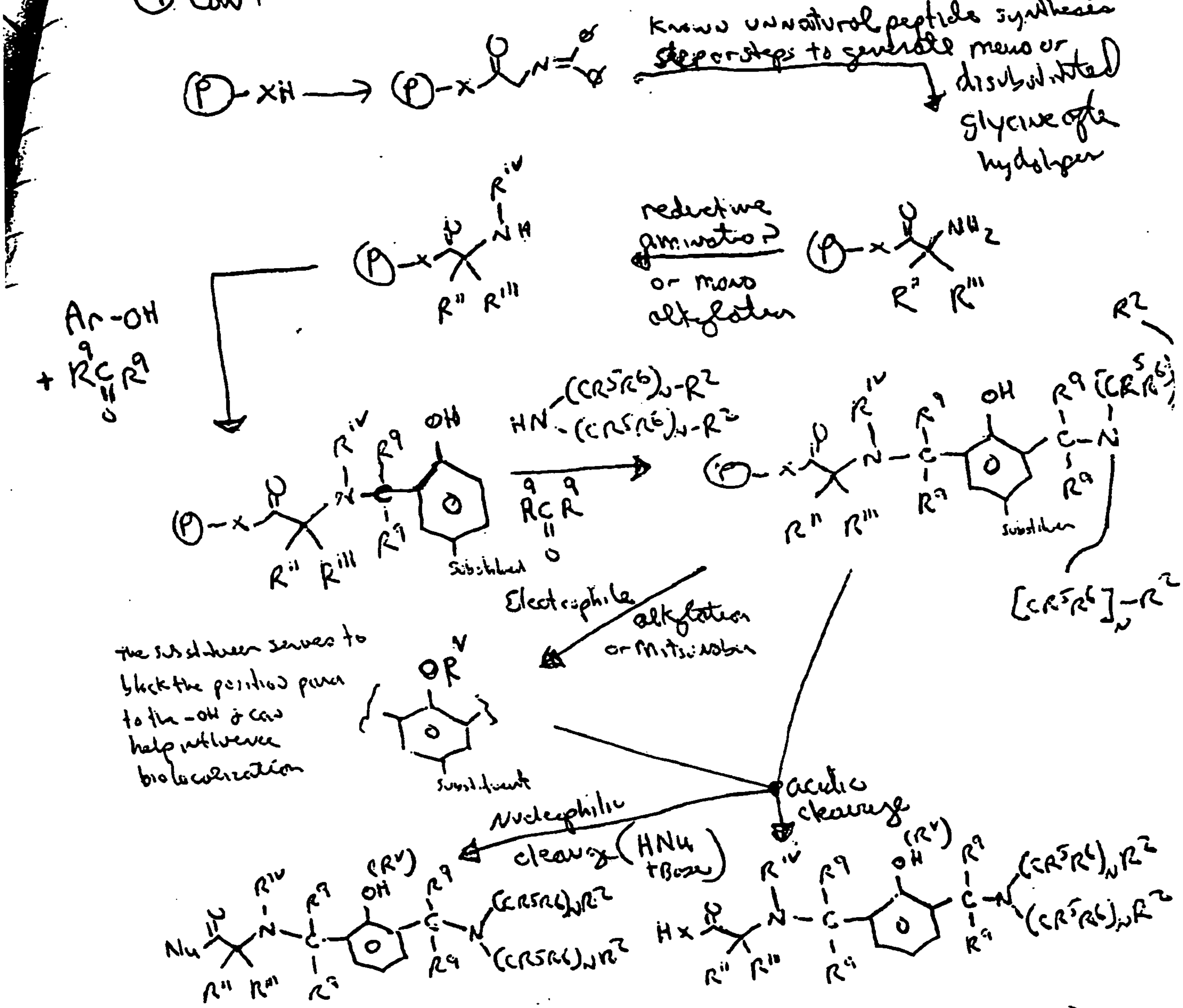

is replaced by:



- other ringsystems that still allow for the chemistry to work
- all above where CE'E" is missing & C attached directly to ring

(4) a method of preparing the above & others (particularly assymetrically substituted phenols or hydroxy aromatics) by starting with one of the chelating arms ($\overset{O}{C}$-CH_2N) attached to solid phase resin as depicted on next page. The advantage to this approach is that assymetrical arms can be elaborated and any aromatic alcohol with ortho positions open should work to give novel compounds

(4) Con't



- R'', R''' are derived from any electrophile that can react with a carbanion
- R^{IV} is derived from an alkylating agent that alkylates a primary amine or an aldehyde/ketone that can be reductively alkylated with a primary amine
- $R^9\overset{O}{C}R^9$ Is an aldehyde or ketone that can react with aromatic alcohols in a Mannich reaction. This can also include chelating groups (ie $H\overset{O}{C}COOH$)
- R^V is derived from an alkylating agent that will react with an aromatic alcohol — an acylating agent or an alcohol that reacts under Mitsunobu conditions to give an ether.
- The other R's are whatever is chemically compatible or as defined in US 5342925 ... in a Mannich Rx

IDEAS ON SYNTHESIS OF NOVEL CHELATORS (CON'T)

(4) CON'T

- R^2 is also envisioned to be a coordinating group other than -COOH, such as but not limited to $-\overset{O}{\overset{\|}{C}}N-OH$; $-\overset{O}{\overset{\|}{P}}(OH)_2$; $-\overset{O}{\overset{\|}{P}}(OH)-OR$; $\overset{O}{\overset{\|}{P}}(OH)-R$; $-ArOH$

(5) It is envisioned that the above novel chelating agents can be complexed with metal ions and these resulting complexes will be useful for diagnostic and therapeutic human medicine depending on what the metal ion is. Especially of interest is Sm-153, Ho-166, Y-90, Dy165, Gd-159, Lu-177, In-111, In-115m, Yb-175, Sc-47, Fe-52, Re-186, Re188.

(6) The $R^9\overset{O}{\overset{\|}{C}}R^9$ is envisioned in the simplest case to be formaldehyde ($H\overset{O}{\overset{\|}{C}}H$) but is also envisioned to be $H\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}OH$, $H\overset{O}{\overset{\|}{C}}-R$, and $R-\overset{O}{\overset{\|}{C}}-R$ where the R groups can be different imparting desirable stereochemical features in the molecule

(7) The metal complexes of the novel complexes describe here are envisioned to be useful in metal-ligand ratios of 1:1 up to several hundred fold excess of ligand (ie 1:300)

(8) The various substituents in the above chelators & hence complexes that are not involved in direct electron donating to the metal ion are chosen & optimized to positively influence the target to nontarget ~~[illegible]~~ ratios & hence improve the therapeutic ratios.

DD/$

PTO/SB/95 (02-2000)
Approved for use through 9/31/2002. OMB 0651-0030
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure Document Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R Garlich
Title of Invention: TUMOR-LOCALIZATION USING INTEGRIN-BINDING MULTIVALENT CONSTRUCTS

Enclosed is a disclosure of the above-titled invention consisting of 3 sheets of description and ______ sheets of drawings. A check or money order in the amount of $10.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

328 West Columbine Lane
Address

Joseph R. Garlich
Typed or printed name

Westfield IN 46074

[redacted]
Date

City, State, Zip

NOTICE TO INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be ... tent application or by a separate letter filed in a pending application. Unless it is desired ... yond the two-year period, it is not required that it be referred to in the patent application.

DISCLOSURE DOCUMENT NO.
481921
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

... sidered to be a "grace period" during which the inventor can wait to file his/her patent ... be recognized that in establishing priority of invention an affidavit or testimony referring ... h diligence in completing the invention or in filing the patent application since the filing ...

... iligence in completing the invention" or "reduction to practice" under the patent law or ... ors are advised to consult with an attorney or agent registered to practice before the ... tered to Practice Before the United States Patent and Trademark Office, is available ... ngton, DC 20402. Patent attorneys and agents are also listed in the telephone ... ties have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

[redacted] ... have been understood and witnessed by persons and/or notarized are other examples of evidence which may ...

... ctions of patents and patent-related ... such as General Information Con... the location of the PTDL closest to ... ies of the Official Gazette, or call the ... To ensure assistance from a PTDL ... and hours.

... Disclosure Document Deposit Requests ... complete, including gathering, preparing ... the individual case. Any comments on ... ed to the Chief Information Officer, U.S. ... ES OR COMPLETED FORMS TO THIS ...

DAWN L. GARLICH
JOSEPH R. GARLICH
328 W COLUMBINE LANE
WESTFIELD, IN 46074

DATE [redacted]

PAY TO THE ORDER OF Commissioner of Patents & Trademarks $10 00/100
Ten and 00/100 DOLLARS

National City
National City Bank of Indiana

Disclosure Document
Joseph R Garlich

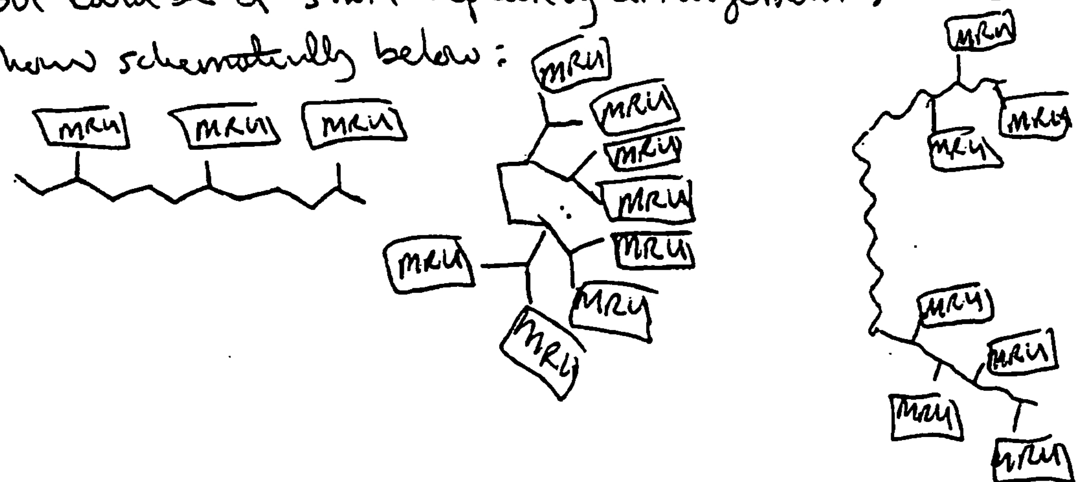
[redacted]

TUMOR LOCALIZATION USING INTEGRIN-BINDING MULTIVALENT CONSTRUCTS by Joseph R Garlich [redacted] P2of3

1) Continued

the in vivo retention. The MRU is the molecular ~~recept~~ recognition unit that has a high affinity for the in vivo receptor sequence or moiety. The arm/linking $\boxed{MRU}$ with the core © can be any of several linear or grafts such as polyol, polyamine, polyamide, peptide, polyaminimide, polyunnatural peptides or any other covalent organic collection that allows spacing between the core & $\boxed{MRU}$ & allows the MRU's to be spacially arranged for maximum binding in a multivalent sense to the in vivo receptor.

2) as in 1 except the core is not a cyclic arrangement but could be a short repeating arrangement such as shown schematically below:



3) 1 & 2 above where the MRU is a peptide or cyclic-peptide known to have selective affinity for the $\alpha_V B_3$ integrins

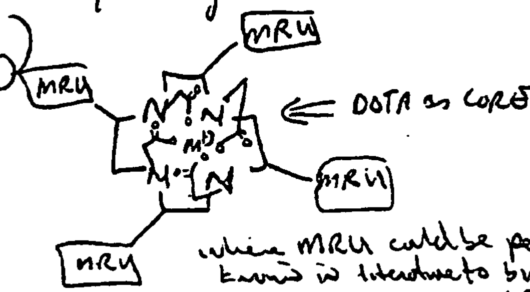
4) 1,2,3, above where the MRU is an analog or mimic of the salient binding points of known peptide or cyclic-peptides known to have selective affinity for the $\alpha_V B_3$ integrins.

Tumor Localization using Integrin-Binding Multivalent Constructs by Joseph R Garlich 10/26/00 P3 of 3

5) 1,2,3,4 above where the core contains a bound radio isotope which can effect diagnostic or therapy of a tumor in vivo

6) 5 above where the core is based on a kinetically Inert macrocyclic lanthanide complex such as DOTA or PCTA and derivatives/analogs.

For examples (non limiting)



where MRU could be peptides known in literature to bind $\alpha_v\beta_3$ integrins

7) 1,2,3,4 above where the radioisotope(s) is (are) bound to the arm linking the core to the MRU

8) 1,2,3,4 above where the radioisotope(s) is (are) bound to a part of the MRU or the organic moiety binding the radioisotope is a critical part of the MRU in assisting with presenting the recognition units? individual binding moieties in spatially organized fashion (see document disclosure 468877 for ideas on what the individual MRU's could be)(and an more definition of what a MRU is envisioned to be)

9) all of the above where MRU is a peptide or peptide mimic containing the Arg-Gly-Asp (so called RGD) sequence.

10) 9 above where the peptide is cyclo-(-Arg-Gly-Asp-D-Phe-Val-) or an analog that is capable of covalent linkage connecting it to the linker back to core structure without destroying activity.

11) all of the above where aminimide moieties are included to improve the in vivo performance of the construct.

DD P

OIPE [redacted] 1999 PATENT & TRADEMARK OFFICE

PTO/SB/95 (06-1999)
Approved for use through 5/31/2002. OMB 0651-0030
Patent and Tradema... U.S. DEPARTMENT OF COMMERCE

Under the paperwork Protection Act of 1995. No persons are required to respond to a collection of informat...

DISCLOSURE DOCUMENT NO.
486682
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

Disclosure Document Deposit Request

Mail To:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. GARLICH
Title of Invention: Chelate Scaffolds (CHELABODIES) in TUMOR TARGETING

Enclosed is a disclosure of the above-titled invention consisting of 10 sheet(s) of description and — sheet(s) of drawings. A check or money order in the amount of $ 10.00 is enclosed to cover the fee. (37 CFR 1.21).

The undersigned being named the inventor of the disclosed invention, requests the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor(s)

328 West Columbine Lane
Address

Joseph R GARLICH
Typed or printed name

[redacted]
Date

Westfield IN 46074
City, State, Zip

NOTICE TO INVENTORS

It should be clearly understood that a Disclosure Document is not patent application, nor will its receipt date in any way become the effective filing date of the later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period, The Disclosure Documents may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the PTO retain the Disclosure Document beyond the two-year period. It is not required that it be referred to in the patent applications.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the disclosure document.

If you are not familiar with what is considered to be "diligence in completing the Invention" of "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the PTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys, which may be consulted.

...ld more than one year prior to the filing of

...of evidence, which may also be used to

...ts and patent-related reference materials ...ng Patents are available at the PTDLs, as ...plete listing of all PTDLs that appears on ...-9199 (800-786-9199) or 703-308-HELP ...bout its collections, services and hours.

...nt Requests. Confidentiality is governed by ...itting the completed Disclosure Document ...o complete this form and/or suggestions for ...eeree, Washington, D.C. 20231. DO NOT ...0231.

DAWN L. GARLICH 02/99
JOSEPH R. GARLICH
328 W COLUMBINE LANE, 317-581-1635
WESTFIELD, IN 46074

163
20-6/740 370

[redacted] DATE

PAY TO THE ORDER OF Commissioner of Patents & Trademarks $10 — $\frac{no}{100}$

Ten and $\frac{no}{100}$ DOLLARS

VALUEPAK

NationalCity.
National City Bank of Indiana
Indianapolis, Indiana

FOR Chelate Scaffolds

Joseph R Garlich

[redacted] 0163

P2

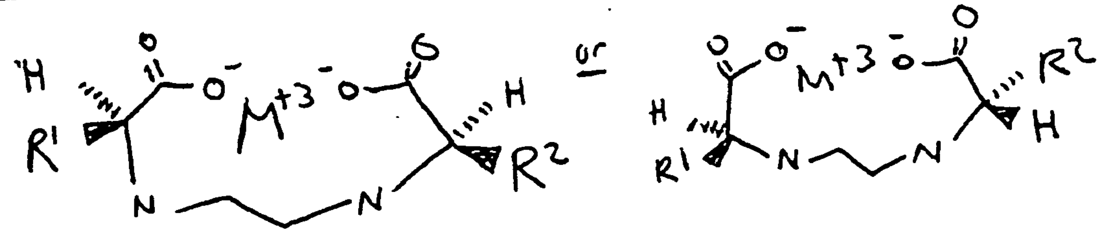
From Joe Garlich

[redacted]

Page 1 of

Kenny: The objective is to have a carboxylic acid group and a guanidine group of of the complex in such a way that they mimic the aspartic COOH and arginine $-NHC(=NH)-NH_2$ groups as define spatially by the cyclopeptide you also modeled. The literature suggests that the distance between the B-carbons of the Arg & Asp groups should be less then 7 Å. I also envision adding in a spacer group (as simple as a methylene group to as large as an aromatic spacer) to the -COOH group or the guanidine group to help achieve proper spacing. Below are some sketches to help communicate some of the possibilities: (only Part of Complex shown for clarity!)

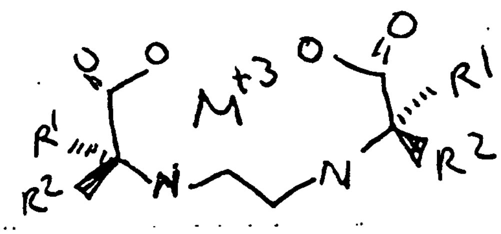
Arm-Arm Attachment:



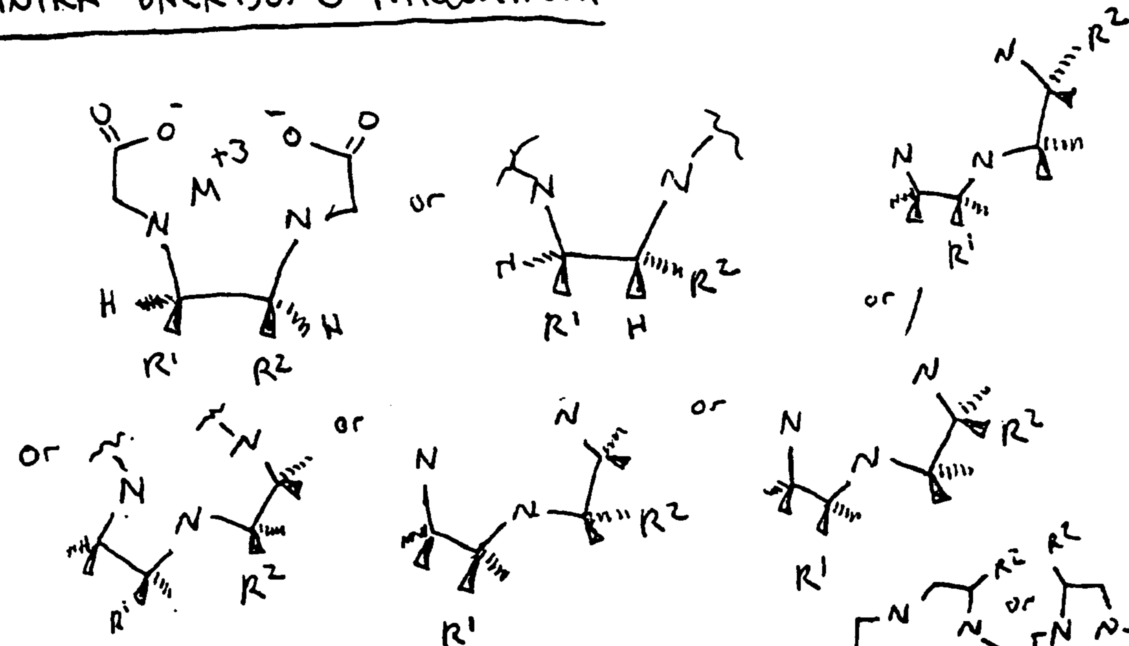
Shown is attachment to acetate arms of adjacent Nitrogens (1,4 of 1,4,7,10-tetraazacyclododecane) but might be able to achieve with accross the ring acetate arms (i.e. acetate arms on 1,7-Nitrogens) substitutions

CONFIDENTIAL

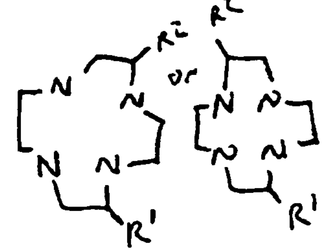
~~Arm~~ Intra-Arm Attachment:

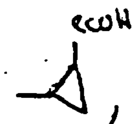
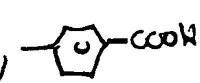


INTRA-BACKBONE Attachment:



also want to check accross ring possibilities:

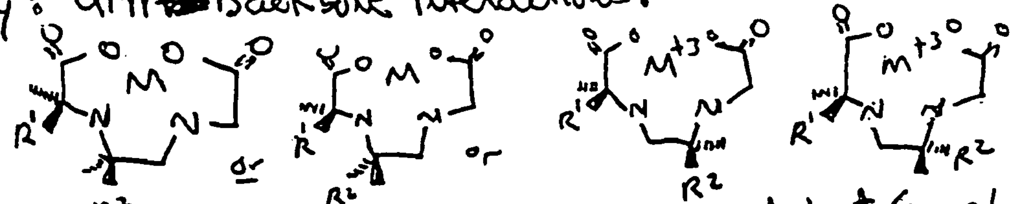


So let R' be $-COOH$, $-CH_2COOH$, , $-C{\equiv}C-COOH$,  etc

(ie: use simple spacers to move out

Likewise let $R^2 = -NH\overset{NH}{\overset{\|}{C}}NH_2$, $-CH_2NH\underset{O}{\underset{\|}{C}}NH_2$, etc

Lastly: Arm ~~-~~ Backbone Interactions:



also possible to look at arm-backbone more distant (ie R' or alkylate at N' with R^2 or ring at position 5, 6, 8, 9 etc.

AgroSciences and has experience in all aspects of combinatorial chemistry-automation, solid-phase and solution phase synthesis, analytical instruments and methodology.

Co-Investigator; Professor Mark A. Green has a background in inorganic chemistry and 18 years of productive research experience in the design, synthesis, and evaluation of new metal-based radiopharmaceuticals. His group is internationally recognized for their efforts in development and pre-clinical testing of low-molecular-weight copper radiopharmaceuticals for imaging with positron emission tomography. For tumor imaging, his group has also pioneered efforts in tumor targeting with low molecular weight folate-chelate conjugates that target a tumor-cell-membrane-associated receptor for folic acid. In addition, they have developed and evaluated an extensive series of monocationic gallium radiopharmaceuticals that are substrates for transport by the MDR1 P-glycoprotein involved in tumor multidrug resistance.

Project Coordinator; Carla J. Mathias brings a background in zoology and chemistry to this project, along with 21 years experience in the design, synthesis, pre-clinical testing, and clinical evaluation of new radiopharmaceuticals. She is experienced in techniques of radiochemical synthesis and analysis, as well as the development and application of animal models for assessment of new radiopharmaceuticals. Her experience includes synthetic, animal, and human studies related to the evaluation of radiolabeled platelets and white cells, radiolabeled antibodies, 18F-labeled estrogen receptor ligands for imaging breast tumors with PET, generator-based PET perfusion tracers, and low molecular weight radiopharmaceuticals targeted to tumor-associated receptor systems.

C nsultants; Dr. O'Donnell pioneered the area of unnatural peptide synthesis which serve as key intermediates in the synthetic aims of this proposal. His interaction will be extremely valuable in achieving the synthetic goals. Dr. Durden, MD, Ph.D. has extensive experience and expertise in vascular biology and integrins. He is an expert in signaling transduction and has much valuable experience in biochemical assays in this area.

D RESEARCH PLAN:

Experimental Plan Stage A & B Rationale and Introduction

Given the drawbacks and approaches described above in the Background section it would be desirable to treat cancers that are highly expressing $\alpha_v B_3$ integrin by a small nonpeptide molecule that 1) possesses a built-in chelating agent complexed with a therapeutic radioactive metal ion in a stable fashion and 2) the resulting nonpeptide metal-ligand molecule possesses a high affinity and selectivity to the $\alpha_v B_3$ integrin. We propose to achieve this with conservation of atoms by using the chelating agent moiety itself as the template upon which to place the $\alpha_v B_3$ integrin binding moieties in a spatial arrangement that mimics the well known $\alpha_v B_3$ integrin antagonist c(RDGfV). The synthesis involved in this approach is detailed in Stage A below. Expanding on this approach is our proposed design to use the chelating agent as the platform from which to tether multiple copies of a selective $\alpha_v B_3$ integrin-binding moiety such as c(RDGfV). This multivalent approach (Stage B), a relatively new concept and not yet applied to integrin binders, will be approached combinatorially to find the optimum distances between the multiple copies of the binding moiety and to study the effect of different spacing groups on the binding of the resulting construct with integrins. The astute reader will recognize after examining the generic schemes that there is some crossover from Stage B into Stage A in that some of the members of Stage A can contain multiple copies of presented binding moieties. This is not an intent to confuse the reader but reflects the great flexibility built into the synthetic approaches.

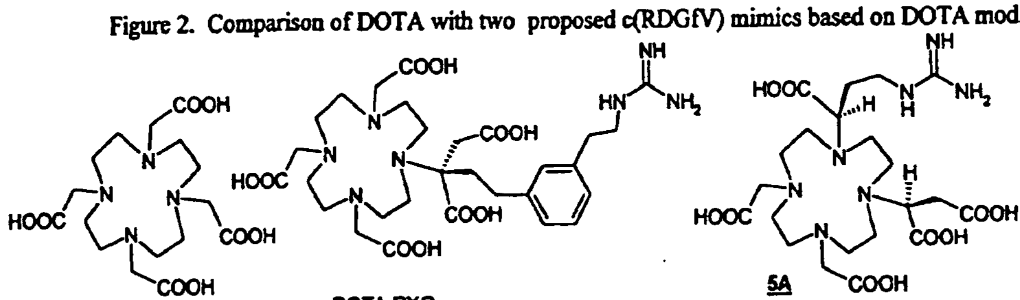
Synthesized molecules that mimic the binding of monoclonal antibodies are called chemobodies[35]. We have coined the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding. Compounds described in both Stage A and Stage B fit into this new category of chelabodies.

Research Plan Stage A: Preparation of RDG Mimics Based Upon Macrocyclic Complexes (Chelabodies)

The chelating agent DOTA, 4 (1,4,7,-10-tetraazacyclododecane-tetraacetic acid), is well know to form kinetically inert complexes with the lanthanides[28] and the resulting complexes are considered conformationally ridgid[29]. The resulting complexes are overall negatively charged at physiological pH when complexed with a trivalent metal ion. The attractiveness of a complex utilizing lanthanides as the metal ion is attributable to the variety of radioactive lanthanides in use in nuclear medicine ($^{153}Sm^{+3}$, $^{90}Y^{+3}$, $^{166}Ho^{+3}$) with differing half-lives and beta-particle energies. The lanthanides tend to be quite similar in their complexation chemistry so that the design of one system may allow the use of any one of several therapeutic radioactive lanthanide metal ions (ie thus more flexibility in choosing the proper radioisotope based upon biological half-life). It should be noted that the Principal Investigator has extensive experience (synthesis, complexation, and radiochemistry expertise) with lanthanides and macrocyclic chelating systems that has led to one commercial drug (Quadramet) and one drug in Phase III clincal trials (STR being evaluated by NeoRx Corporation). Another attractive feature of the DOTA chelator system is its widespread use in clinical MRI imaging agents and bifunctional chelating agents for attaching radioactive lanthanides to monoclonal antibodies for use in humans.

An inspection of molecular models of DOTA complexes indicates that DOTA is similar in size to the peptide ring $\alpha_v B_3$ integrin antagonist c(RDGfV). This led us to the idea that suitable c(RDGfV) mimics could be prepared by judicious subsititution patterns on the DOTA backbone. For example, molecular modeling indicates that structure 5 (DOTA-RXG)when complexed with Y^{+3} would place the guanidine and carboxylic acid in a similar spatial arrangement as that found for the guanidine of the arginine and the carboxylate of the aspartic acid residues in c(RDGfV)[29]. Likewise, from modeling estimates structure 5A (upon complexation with Y^{+3}) appears to also satisfy the spatial requirements of the binding moieties of c(RDGfV)[29]. Stucture 5 represents a single arm attachment and structure 5A represents adjacent chelating arm modifications. It should be noted that modeling indicates that similar achievement of a c(RDGfV mimic using modifications of acetate arms that are not adjacent would be difficult unless extremely large and conformationally floppy spacer groups are used. Thus our effort will be focused initially on 5 and 5A and their analogs.

Figure 2. Comparison of DOTA with two proposed c(RDGfV) mimics based on DOTA modifications.



DOTA 4 | DOTA-RXG 5 | 5A

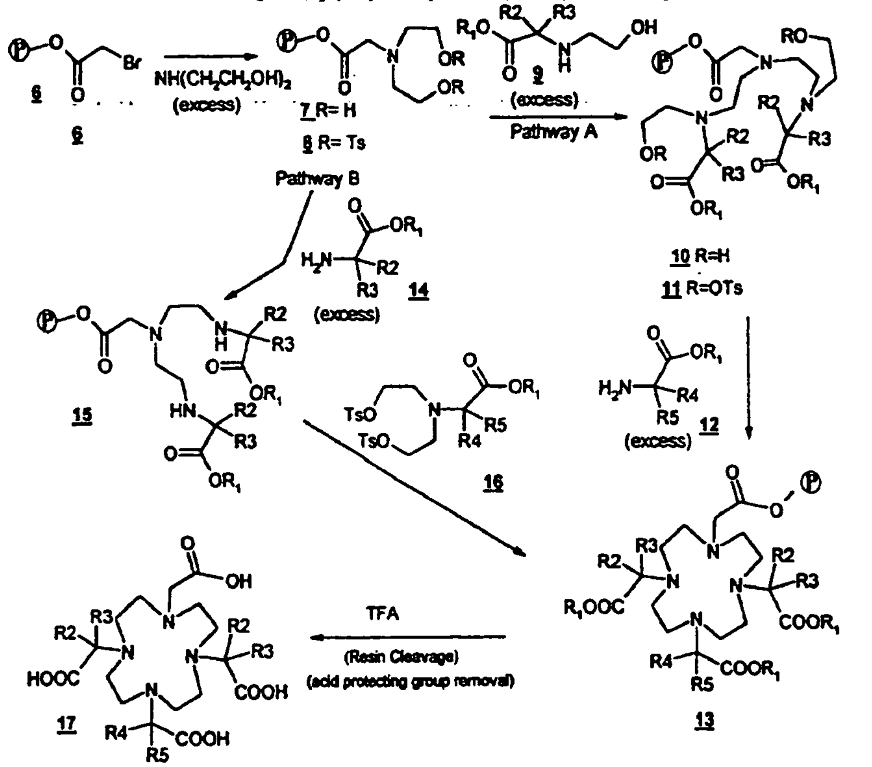
There are numerous other possible substitutions on the acetate arm besides those shown in 5 and 5A which could restrict rotation even further to provide additional preorganization to mimic c(RDGfV). Additionally there are many additional groups that can serve as carboxylate mimics and guanidine mimics. Our plan is to prepare a library of compounds similar to 5, guided by molecular modeling, via the solid-phase combinatorial chemistry route proposed in Figure 3.

In Figure 3 the circled P represents the solid phase resin, Wang resin in this case. However, the use of Rink amide resin is also to be evaluated which would give a DOTA-based chelator wherein one of the chelating acetate arms is a -CH2C(O)NH2 group upon cleavage from the resin. These types of chelators are known and while they are not as stable as DOTA they are stable enough for *in vivo* use[29]. An additional advantage of this monoamide from Rink amide resin would be that the resulting complex with trivalent lanthanides would give a neutral complex core molecule. This could have important *in vivo* biodistribution effects which will be studied.

The synthetic scheme (Figure 3) to prepare these molecules illustrates two pathways to get to the same desired substituted DOTA chelator, 17. Both pathways will be examined and each will require significant

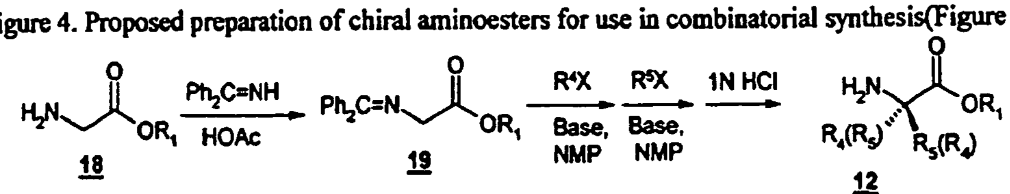
optimization work. These efforts would represent the first on-resin synthesis of the medically important tetraazacyclododecane ring system. We thus feel that this work, even if ultimately unsuccessful in the biological evaluation, will be a welcome and exciting combinatorial chemistry methodology advance in the area of chelation based inorganic medicinal chemistry. By using R2=R3=H the synthesis as shown in Figure 3 simplifies to only one chelator arm substituted with two moieties. The stereochemistry is not shown in Figure 3 but the use of the proper enantiomer of 12, which we plan to isolate and obtain in each instance, will deliver the desired stereoisomer as shown in structure 5.

Figure 3. Proposed solid-phase synthesis of 5 (R2=R3=H; R4=CH_2COOH; R5= CH_2CH_2-p(Ph)-NH(C=NH)NH_2) as a single member of a combinatorial library.



The key building unit to get to structures like 5 via the route shown in Figure 3 is a chiral unnatural amino acid derivative. A diverse collection of these disubstituted glycine derivatives can be prepared in solution phase or solid phase by the UPS (unnatural peptide synthesis) route pioneered by O'Donnell who is serving as a consultant on this proposal[31,32]. This procedure is shown in Figure 4 and lends itself to automation[33]. It is anticipated that the different enantiomers resulting in Figure 4 will be separated using chiral chromatography. There are methods to perform the chemistry in Figure 4 wherein either R4 or R5 is hydrogen with significant stereoselectivity (80-90% ee) but our criteria for purity (>95%) requires that we perform a chiral separation at this stage. This will be performed using HPLC methodology.

Figure 4. Proposed preparation of chiral aminoesters for use in combinatorial synthesis(Figure 3).

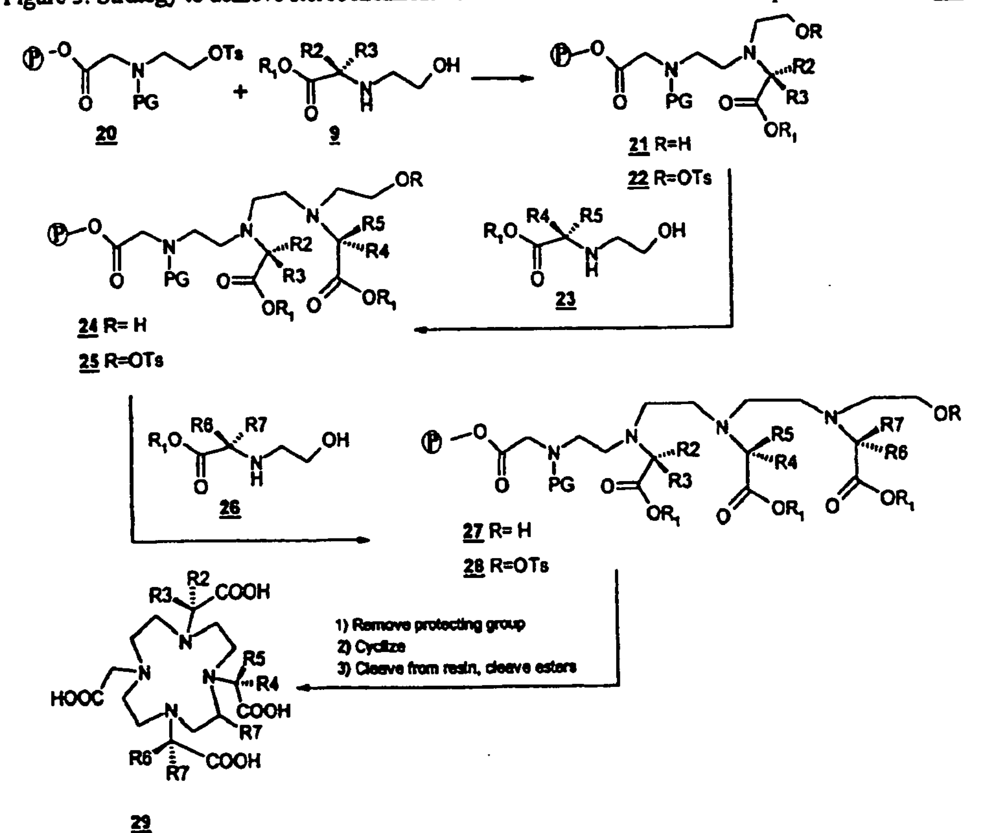


With the inputs 12 (and 14 which can be the same or different from 12, derived from the same chemistry) in hand then the library production protocol based on structure 5 can be developed. Because of the way the

synthesis is developed it is possible to make an analog of 5 where each of the three acetate arms contain one copy of the RDG mimic structure by making 12 and 14 the same aminoester. This trivalent species, by benefit of compact presentation of three copies of the RDG mimic strucure, could possess some interesting properties. There is more discussion later regarding this multivalent approach in the research plan stage 2 discussions.

In order to access desired target molecules such as 5A a different synthesis route is needed since two identical molecules of aminoester are incorporated in either pathway A or pathway B in Figure 3. This uncontrollable dual incorporation precludes introducing the needed stereochemistry at both sites, i.e. only one acetate substitution pattern will have the correct configuration. To address the desired access to molecules like 5A and to give complete control over the stereochemistry of all 6 substituents on the chelating acetate arms the synthetic protocol shown in Figure 5 will be evaluated. The amino alcohols 9, 23, and 26 will be prepared from the corresponding unnatural amino esters prepared by the method shown in Figure 2 and purified to get the single isomer. The preparation of these aminoalcohols could make use of resin bound ethylene glycol wherein the amine of the amino ester (such as 12) displaces the activated non-resin bound hydroxyl of the ethylene glycol. The PG (protecting group) on the nitrogen of Figure 5 will be determined after some preliminary work is

Figure 5. Strategy to achieve stereochemical control at each chiral acetate arm position such as 5A.



performed to ensure othogonal stability but likely will be a group such as FMOC, NOSYL, or trifluoracetamide.

These proposed chelator scaffolds (chelabodies) addresses all of the shortcomings described previously for a tumor neovasculature seeking agent. The positive attributes for this system are 1) nonpeptide in nature so not prone to metabolism; 2) incorporates a kinetically inert lanthanide complex which allows for a potential range of radioisotopes having varied particle energies and half-lives and yet produced commercially (Sm-153, Ho-166, and Lu-177); 3) rigid backbone (cyclododecane ring system locked into place upon chelation) upon which to place appropriately spaced recognition/binding groups; 4) the complex containing the toxiphore (radioactive metal ion) is part of the core rigidifying structure so no additional conjugation chemistry is required, i.e. the compound from screening will not need to be further modified to label with a radioactive isotope;

168

Research Plan Stage B:

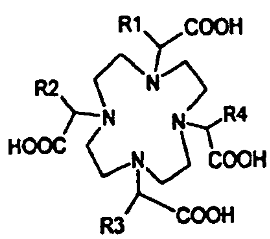
Preparation of Extended Multivalent RDG Mimics Based Upon Macrocyclic Complexes (Chelabodies)

Monoclonal antibodies are known for their exquisite selectivity and high binding affinity. These attributes arise in part because antibodies are divalent and in some cases multivalent in their binding with proteins or receptor surfaces. Nature has used multivalent binding to overcome weak binder in order to make strong attachments[35]. Multivalency, simultaneous attachment of two or more binding sites on one molecule (drug) to multiple receptor sites on another (cell surface), is a new approach to drug design according to George M. Whitesides of Harvard University[35,36]. This multivalent approach has not yet been applied to ligands aimed at binding the integrins although Burgess has disclosed a cyclic sequence, c(RDGRGD), that could be considered a dimer of RDG[37]. Surprisingly this ligand possessed excellent selectivity and antagonistic activity towards α_vB_3 integrin.

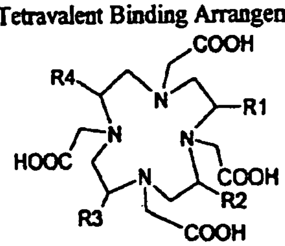
This area of multivalent drug design is where the term "chemobody" has been coined to describe synthesized molecules that mimic the binding of monoclonal antibodies[35]. We are proposing the term "chelabodies" to describe chelates (metal-ligand complexes) that mimic the binding of monoclonal antibodies. Thus, chelabodies represent a subset of chemobodies wherein the chelate is a critical design feature that causes arrangement of the binding motifs in the appropriate spatial arrangement to give antibody-like multivalent binding.

Research plan stage B comprises the design and evaluation of multivalent presentations of α_vB_3 integrin antagonists based on the DOTA template. This is illustrated conceptually in Figure 6 where either four substitutions are made on the chelating arms (30) or situated around the macrocyclic ring (31). We have also considered the possibility of a mixed species where some substitution is on the acetate arms and some is on the backbone carbons but no compelling reason exists to pursue this approach over the other two described here in more detail. Given the resource available in this proposal we will put our effort in the arm substituted system (30) since that approach takes advantage of the chemistry worked out in research plan A. The focus of this proposal is for the R groups to contain, preferably at their terminus, a moiety that is an α_vB_3 integrin

Figure 6. Conceptual design of Chelabodies Based on DOTA-type Chelating Agents Presenting a Tetravalent Binding Arrangement Aimed at α_vB_3 Integrin Antagonism.



30



31

R1,R2,R3,R4= Z-M

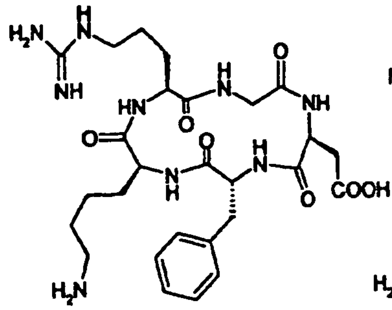
Z= linker/spacer of varible length, shape, flexibility

M= RDG mimic that selectively antagonizes avb3 intetrin

antagonist. The ideal terminal group would be one that induces internalization of the bound ligand into the cell and compounds will be tested for this property (see biological assay section). In order to prove the concept involved here we first will use known antagonists at the terminal binding positions. For example the known antagonist c(RDGfK) (32) has been described and is amenable to capping off the "R" arms to provide a suitable multivalent antagonist construct. This compound will either be synthesized in-house or custom prepared for CCTI outside of the budget requested here. The linker/spacer arms can be similar to those described in the literature for multivalent constructs, some of which are illustrated in Figure 7. One basic linker arms idea is to react carboxylic anhydrides with a nucleophile such as nitrogen on the arm stub and then couple a diamine with the resulting free carboxylic acid. This procedure is amenable to solid-phase synthesis to prepare arms that are all the same[38,39]. Applying this strategy to the compounds of Figure 4 and Figure 5 requires only that some of the substituents (R2, R3, R4, R5, R6, R7) on the arm building blocks (9, 12, 14, 16, 23, 26) contain a masked electrophile (to react with amines for example) or nucleophile (to couple with carboxylic acids for example) that

can be deprotected and then elaborated into a linke/spacer module for endcapping with antagonists such as **32**. This approach would work via the chemistry outlined in Figures 4 and 5 to give essentially trivalent constructs (i.e. one per each substituted chelator arm). There is no convenient method to get to a fully symmetrical tetravalent system using solid phase methodology so solution phase methods will be examined. It is apparent that there are a large number of possible constructs that could be prepared varying the nature and length of the arms.

Figure 7. Proposed Endcap Moiety for $\alpha_v B_3$ Integrin Antagonist in a Multivalent Construct and Examples of Linker/spacer Modules.



32

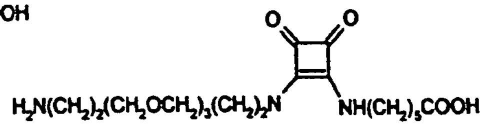
c(RDGfK)

cyclo-(-Arg-Gly-Asp-D-Phe-Lys-)

$NH_2CH_2CHCH_2$-$(OCH_2CH_2)_3$-CH_2NH-$COCH_2CH_2COOH$

(ref 38)

33



(ref 39)

34

Our approach is to prepare a combinatorial library of such constructs and to assess their biological binding and performance (*in vitro* binding and whole cell assays) to determine if improvements in tumor cell localization are possible.

Research Plan Biological Evaluations:

Assay-In Vitro: The ELISA-type in vitro testing for competitive binding of test ligands with $\alpha_v B_3$ integrin is well established as are the methods to obtain the needed starting materials; vitronectin, $\alpha_v B_3$ integrin, firbrinogen, and $\alpha_{IIb} B_3$ integrin[19, 22, 27, 41, 42, 43]. The procurement of some of these will be at CCIT's cost outside of the budget proposed in this application. Briefly, the solid-phase competitive displacement *in vitro* assay test comprises; 1) coating 96-well plates with $\alpha_v B_3$ integrin receptor (or $\alpha_{IIb} B_3$ integrin receptor to determine selectivity), 2) washing sequence including 1% BSA, 3) exposure to various concentrations of test compound containing biotinylated vitronectin (or biotinylated fibronectin)[19] for 2 hours, 4) washing sequence, and finally 5) detection of biotin present using reporter-labeled anti-biotin antibody. This testing will be performed on nonradioactive metal ion complexed with our newly synthesized compounds so that it can be performed in a medium-throughput mode at the Purdue Center for Combinatorial Chemical Biology.

Assay- In Vitro Whole Cell Internalization Studies: A recent method has been described to determine internalization of integrins which are thought to occur via endocytosis[44]. Our approach will not necessarily measure internalization (which requires anti-ligand antibodies) but will expose integrin expressing cells to our synthesized ligands and then determine the degree of binding by aggressive exposure to competitive ligand and various washes. Since all of our molecules chelate radioactive metal ions these radioactive metal complexes will be easily determined to be either cell associated, or easily removed. The ultimate location of our ligands is less important than ensuring that the antagonists stay bound to the cell surface so that *in vivo* they are able to deliver the desired radiation dose.

Animal Studies: *In vivo* evaluation of the best *in vitro* active compounds. The animal testing we will perform will follow those most recently published in the area of nuclear medicine[19]. These animal results using human tumors implanted into immune-compromised mice will provide biolocalization data. We will not be measuring antitumor effects as the animals will be sacrificed to quantitate the tumor and normal tissue uptake. The tumors and cell line we will be using is the melanoma line WM164 available from ATTC.

P10

Specific Goals/Accomplishments Expected for Phase I Year 1:

1 Perform modeling of complexes (chelabodies) that will mimic neovasculature targeting peptide-receptor binding interactions via substitution patterns on a DOTA-lanthanide complex scaffold..

2 Several virtual libraries of complexes are assessed by molecular modeling of receptor fit to determine synthetic direction have been performed.

3 Synthetic methodology has been developed to create macrocyclic chelator based libraries that are mimics for the c(RDGfV) binding ligand.

4 Binding assays are developed to screen libraries, some libraries have been evaluated and some hits are identified. Also, a whole cell binding assay has been evaluated and implemented.

5 Hits from biological screens are confirmed, identified and synthetic effort to optimize at least some of these hits has been initiated.

6 Confirmed hits from biological screens have been evaluated in tumor bearing mice.

7 Work has begun to evaluate the feasibility of making multivalent constructs. Some constructs will have been prepared.

Specific Goals/Accomplishments Expected for Phase I Year 2:

1 Optimized leads from research plan stage A have been evaluated *in vitro* and *in vivo* and are ready for preclincial studies.

2 Synthetic methodology has been developed for preparing multivalent constructs in research plan stage B.

3 Multivalent construct libraries from research plan stage B have been prepared and hits optimized from *in vitro* and *in vivo* testing to give maximum tumor localization of radiometal isotope.

E HUMAN SUBJECTS- NONE

F VERTEBRATE ANIMALS

1. Athymic mice (~135 per year) will be required for screening each new radiopharmaceutical (that shows promise in *in vitro* studies) to determine the agent's tumor localization *in vivo*. We plan to screen and evaluate 15 new radiotracers *in vivo* per year, using nine animals per compound. The tumor-bearing athymic mice are required for assessment of radiotracer distribution and pharmacokinetics, plus demonstrating that the tumor uptake of tracer is mediated by binding to the $\alpha_V\beta_3$ receptor. In this project we will conduct cell culture studies as a preliminary screen of tracer affinity for the $\alpha_V\beta_3$ receptor, to insure that biological data is only collected from animals in cases where there is a good probability of targeting tumor-vasculature-associated receptors, thereby minimizing animal usage as well as experimental expense. The athymic mice will be implanted with human tumor cells (WM164 human melanoma available from ATCC) using standard aseptic techniques, and housed under aseptic conditions until tumor growth is evident. The mice will then be used for biodistribution studies designed to determine the tissue distribution and pharmacokinetics of the test tracers. The radiopharmaceutical will be administered intravenously *via* the exposed femoral vein (to allow visual verification that the dose is completely delivered into the vein) with the animal under diethyl ether anesthesia. Tissues that will be sampled for quantification of radiopharmaceutical uptake include the tumors, blood, heart, lungs, liver, spleen, kidneys, stomach and intestines, muscle, fat, and brain. For each tracer, data will typically be collected at 2 and 24 hours post-injection, examining 3 animals per time point. An additional 3 animals will be examined at one of these time points after co-administration of the radiotracer with an excess of a known high-affinity $\alpha_V\beta_3$ ligand, in order to demonstrate the expected competitive blocking of radiopharmaceutical uptake in tumor. This blocking study will also implicitly provide a measure of the level of non-specific radiotracer uptake in tumor. If it appears likely to assist in interpretation of the resulting mouse data, biodistribution data will also be collected for ^{64}Cu-PTSM and ^{18}F-FDG in the mouse tumor model(s), allowing direct assessment of the rate of tumor perfusion, and rate of metabolism, respectively. The athymic mouse has been chosen as our primary animal tumor model since it can serve as a host for a variety of human tumor cell lines, and is easy to handle and maintain.

2. The use of animal models for screening potential new radiopharmaceuticals is essential to the development of improved diagnostic imaging agents for use in clinical nuclear medicine. The athymic mouse is

TO: Kenny Lipkowitz

Chemistry Dept.

IUPUI 274-4701

CONFIDENTIAL

From: Joe Garlich

ComChem Technologies Inc.

418-8246

3 pages including Cover Sheet

Faxed 9:45am [redacted]

DISCLOSURE DOCUMENT NO.

473368

RETANED FOR 2 YEARS

THIS IS NOT A PATENT APPLICATION

Mr. Joseph Garlich
328 W. Columbine Lane
Westfield, IN 46074

OIPE JC33

[redacted] Approved for use through [illegible] ...t and Trademark Office; U.S. DEPT

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays ...

PTO-1652 (8/98)

Disclosure Document Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: IDEAS ON TUMOR TREATMENT USING Neutral LIPOPHILIC Complexes

Enclosed is a disclosure of the above-titled invention consisting of 2 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

Joseph R. Garlich
Typed or printed name

[redacted]

328 West Columbine Lane
Address

Westfield
IN 46074
City, State, Zip

NOTICE TO INVENTORS

...stood that a Disclosure Document is not a patent application, nor will its receipt date in any way ... date of a later filed patent application. A Disclosure Document may be relied upon only as evidence ...on and a patent application should be diligently filed if patent protection is desired.

...ll be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be ... is referred to in a related patent application filed within the two-year period. The Disclosure Document may be ... transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired ...sclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

...d should not be considered to be a "grace period" during which the inventor can wait to file his/her patent ...ses of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring ...ust usually also establish diligence in completing the invention or in filing the patent application since the filing ...

...at is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or ...about patent matters, you are advised to consult with an attorney or agent registered to practice before the ... *...eys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available ...of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone ...s. Also, many large cities have associations of patent attorneys which may be consulted.

...ny public use or sale in the United States or publication of your invention anywhere in the world more than one ...ent application on that invention will prohibit the granting of a patent on it.

...ch have been understood and witnessed by persons and/or notarized are other examples of evidence which may ...ority.

...k of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related ... to the public, including automated access to PTO databases. Publications such as *General Information Con-* ...s at the PTDLs, as well as the PTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to ...plete listing of all PTDLs that appears on the PTO's Web site or in every issue of the *Official Gazette*, or call the ...ervices at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To ensure assistance from a PTDL ... to contact a PTDL prior to visiting to learn about its collections, services, and hours.

...llection of information is used by the public to file (and by the PTO to process) Disclosure Document Deposit Requests. ...USC 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, ...Disclosure Document Deposit Request to the PTO. Time will vary depending upon the individual case. Any comments on ... to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. ...e, Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ...t Commissioner for Patents, Washington, D.C. 20231.

ck 6301

PTO/SB/95 (06-1999)
Approved for use through 6/30/2002. OMB 0651-0030
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure D cument Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: Ideas on Tumor Treatment using Neutral Lipophilic Complexes

Enclosed is a disclosure of the above-titled invention consisting of 2 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

328 West Columbine Lane
Address

Joseph R. Garlich
Typed or printed name

Westfield

IN 46074
City, State, Zip

[redacted]
Date

NOTICE TO INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the PTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony relating to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the PTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

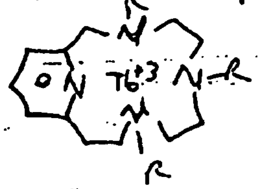
There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related reference materials available to the public, including automated access to PTO databases. Publications such as General Information Concerning Patents are available at the PTDLs, as well as the PTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the PTO's Web site or in every issue of the *Official Gazette*, or call the PTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To ensure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted]

IDEAS ON TUMOR TREATMENT — Page 1 of 2 Joe Gardella

USING Neutral Lipophilic Complexes — Joseph R Gardella

PCTMB complexed with Terbium^{+3} ion has been found by others to localize selectively in colorectal cells (see Analytical Chemistry 1999 vol 71 pp 2607-2615)



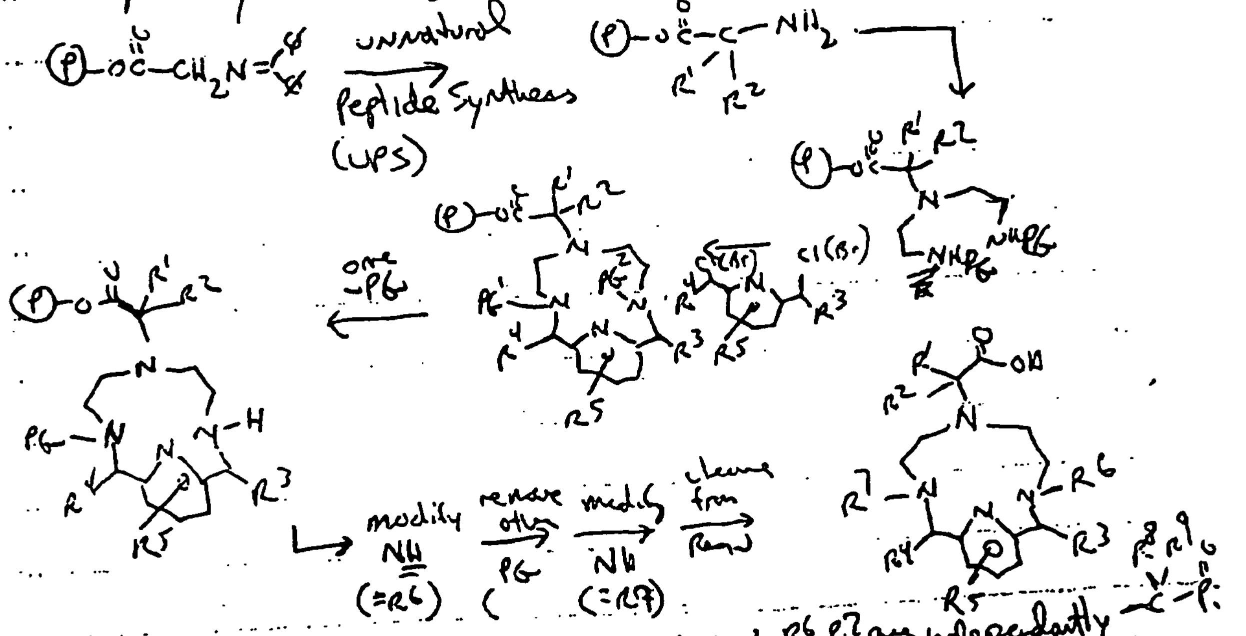
PCTMB

R = $-CH_2P(O)(O^-)-O$

Tumor or abnormal tissue -

I propose that better localizing molecules can be identified using combinatorial chemistry to build libraries & find the ones that complex therapeutically useful metal ions such as $^{153}Sm^{+3}$, $^{166}Ho^{+3}$, $^{90}Y^{+3}$, $^{149}Pm^{+3}$, $^{159}Gd^{+3}$, $^{140}La^{+3}$, $^{177}Lu^{+3}$, $^{175}Yb^{+3}$, $^{47}Sc^{+3}$, $^{175}Yb^{+3}$, $^{142}Pr^{+3}$. I also envision these complexes in diagnostic capacity including metal ions such as Gd^{+3}

1) I propose attachment to solid phase resin thru a carboxylate instead of phosphonate followed by building up the ring & subsequently modifying the ring nitrogens.



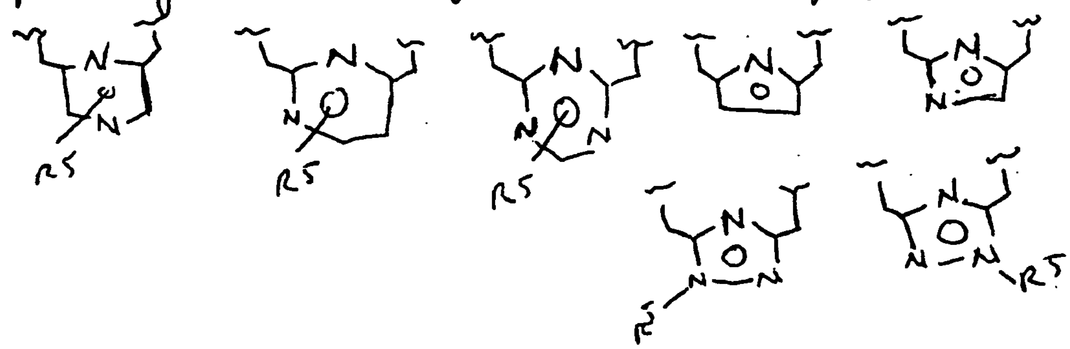
R1, R2, R5, R3, R4 are organic moieties that do not chelate; R6, R7 are independently where R8, R9, R10 do not chelate but are organic moiet

 [redacted]

IDEAS ON TUMOR TREATMENT (CONTINUED) Joseph R. Sachs

2) The ligands of 1 above are envisioned to be therapeutically useful chelating agents ~~tat~~ that will localize ~~to~~ selectively in tumor cells & treat diseases.

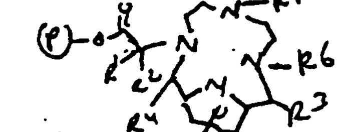
3) The heterocyclic ring of 1 above incorporating R5 can be any number of heterocyclic rings where the substituent(s) R5 are not chelating but positively influence the localization of the complex in the target tissue. For example,



4) The chelating arms of 1 above namely R6 & R7 are optimized for localizing selectivity and are monovalent & single-charged. They can be phosphates (as half esters as indicated) or carboxylates with a substitution on the alpha carbon that allows the group to mimic the desired spatial & lipophilic nature of the PCTMB ester group.

ie R6 & R7 composed alternatively or independently of

$-\underset{R^{11}}{CH}COOH$; $-\underset{R^{11}\ R^{12}}{C}-COOH$ where R^{11}, R^{12} are non chelating organic moieties of molecular weight less than 250.

5) also envisioned by this process is where the carboxylic arm is on the "side" eg:  nitrogen

6) also envisioned is [illegible] the difference between the nitrogen in the ring

DISCLOSURE DOCUMENT NO

473388

RETAINED FOR 2 YEARS

THIS IS NOT A PATENT APPLICATION

OIPE JC35 PATENT & TRADEMARK OFFICE [redacted]

Approved for use through ... Patent and Trademark Office; U.S. DE... Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displa...

PTO-1652 (8/99)

Disclosure Document Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Mr. Joseph Garlich
328 W. Columbine Lane
Westfield, IN 46074

Inventor(s): Joseph R. Garlich

Title of Invention: Ideas on Alternatives to Ionizing Radiation in Treatment of Medical Diseases

Enclosed is a disclosure of the above-titled invention consisting of 4 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R. Garlich
Signature of Inventor

328 West Columbine Lane
Address

Joseph R. Garlich
Typed or printed name

Westfield

IN 46074
City, State, Zip

[redacted]

NOTICE TO INVENTORS

...ood that a Disclosure Document is not a patent application, nor will its receipt date in any way ...ate of a later filed patent application. A Disclosure Document may be relied upon only as evidence ...n and a patent application should be diligently filed if patent protection is desired.

...be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be ...s referred to in a related patent application filed within the two-year period. The Disclosure Document may be ...s referred to in a related patent application or by a separate letter filed in a pending application. Unless it is desired ...ransmittal in a new patent application or by a separate letter filed in a pending application, it is not required that it be referred to in the patent application. ...closure Document beyond the two-year period.

...should not be considered to be a "grace period" during which the inventor can wait to file his/her patent ...f establishing priority of invention an affidavit or testimony relating ...s of benefits. It must be recognized that in establishing priority of invention or in filing the patent application since the filing ...t usually also establish diligence in completing the invention ...

...t is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or ...bout patent matters, you are advised to consult with an attorney or agent registered to practice before the ...Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office, is available ...f Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone .... Also, many large cities have associations of patent attorneys which may be consulted.

...y public use or sale in the United States or publication of your invention anywhere in the world more than one ...ent application on that invention will prohibit the granting of a patent on it.

...ch have been understood and witnessed by persons and/or notarized are other examples of evidence which may ...rity.

...k of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related ...to the public, including automated access to PTO databases. Publications such as General Information Con... ...s at the PTDLs, as well as the PTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to ...plete listing of all PTDLs that appears on the PTO's Web site or in every issue of the Official Gazette, or call the ...ervices at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To ensure assistance from a PTDL ...to contact a PTDL prior to visiting to learn about its collections, services, and hours.

...ection of information is used by the public to file (and by the PTO to process) Disclosure Document Deposit Requests. ...USC 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, ...Disclosure Document Deposit Request to the PTO. Time will vary depending upon the individual case. Any comments on ...to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. ...S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ...Commissioner for Patents, Washington, D.C. 20231.

CK 6302

PTO/SB/95 (06-1999)
Approved for use through 8/31/2002. OMB 0651-0030
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure Document Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: Ideas on Alternatives to Ionizing Radiation in Treatment of Medical Diseases

Enclosed is a disclosure of the above-titled invention consisting of 4 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R. Garlich
Signature of Inventor

Joseph R. Garlich
Typed or printed name

[redacted]
Date

328 West Columbine Lane
Address

Westfield
IN 46074
City, State, Zip

NOTICE TO INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way
become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence
of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be
destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be
referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired
to have the PTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent
application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring
to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing
of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or
if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the
PTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available
from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone
directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one
year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may
also be used to establish priority.

There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related
reference materials available to the public, including automated access to PTO databases. Publications such as General Information Con-
cerning Patents are available at the PTDLs, as well as the PTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to
you, please consult the complete listing of all PTDLs that appears on the PTO's Web site or in every issue of the Official Gazette, or call the
PTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To ensure assistance from a PTDL
staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted]

IDEAS ON ALTERNATIVES TO IONIZING RADIATION IN TREATMENT OF MEDICAL DISEASES: By Joe Garlich Joe Garlich

Metal ions such as but not limited to iron, manganese, and copper are effective catalysts for the decomposition of peroxides particularly hydrogen peroxide (See for example Inorganica Chimica Acta, 1992 pp 359-367). When this decomposition by metals occurs in the presence of organic substrates (such as in the human or animal body) the hydroxyl radicals produced will oxygenate the organic substrates (known as the Fenton Reaction). This process is presumably what occurs indiscriminately when ionizing radiation (radiation therapy, loose isotope radiopharmaceuticals) is used in Nuclear medicine (see Radiation Biology. book by Alison P. Casarett, 1968, U.S. Atomic energy commiss, p68). There are as yet no known attempts to chemically reproduce the ionizing effects of radiation using targeted oxidant or targeted metal ion species. The proposals below describe my ideas and concepts in delivering a therapeutic dose of oxidizing species selectively to diseased tissues in humans and animals.

i). Use a coordinatively unsaturated phosphonic or poly phosphonic acid complex with metal ions to deliver said metal ions to the skeletal system upon introduction into the body (Just like Quadramet drug localizes or Neoprobe's STR drug approach with radioactive lanthanide metal ions). After skeletal or bone tumor →

(continued) [redacted]

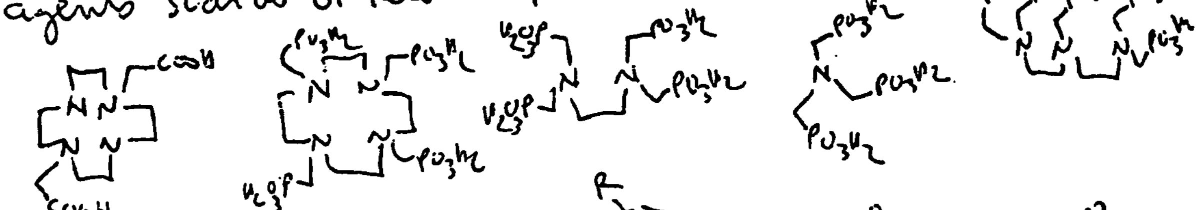
Joe Sadish

localization of the metal complex (such as a Fe^{+3} complex) the peroxide source is then introduced into the body. By timing these two administrations such that any nontarget localized metal ion complex is cleared from the body one can achieve excellent selectivity in the generation of toxic oxidizing species since they will arise only where both peroxide species and metal ion complex are present in significant amounts.

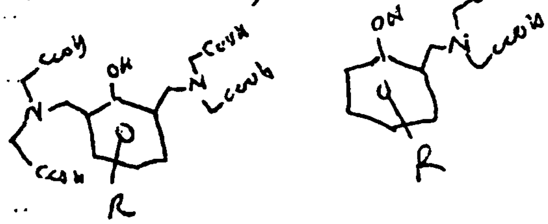
2) The use of #1 above in ablating marrow prior to any kind of bone marrow transplant procedure.

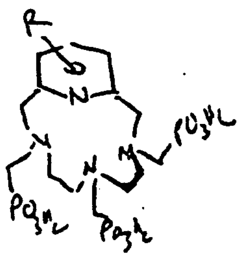
3) The use of #1 above in treating disorders of the bone or bone marrow such as cancer metastases to the bone, Leukemias, Lymphomas, multiple myeloma, sickle cell anemia etc

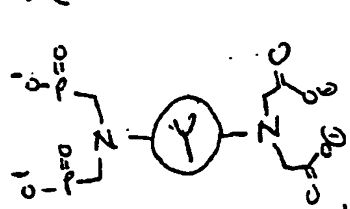
4) #1 where the bone seeking complex is composed of Cu, Fe, or Mn (in various oxidation states) complexed with chelating agents such as or related to those shown below



(overall ⊕ charge)







when Y holds aminocarboxyl metal piece to the phosphonic acid bone seeking piece

or more general:

X —(Y)— Z

X = $-\overset{O}{\underset{}{P}}(OH)_2$ group or groups (ie bone seeking)

Y = Linker to hold X + Z covalently together

Z = Fe^{+3}, Mn^{+}, Cu^{+2} chelating agent

[redacted]

[CONTINUED]

Joe Sarle

5. The peroxide contemplated in #1 above can be any organic or inorganic peroxide that in and of itself is acceptable non-target toxicity such as but not limited to

A) various concentrations of aqueous hydrogen peroxide H-O-O-H

B) Perborate

C) Persulfate

d) Per-phosphoric acid R-P(=O)(-OH)-O-O-H; preferably a polyphosphoric acid with bone seeking properties in and of itself to help target therapy

e) a per carboxylic acid R-C(=O)-O-O-H

The requirement for the peroxide is that it exhibits adequate pharmacokinetics when introduced in bloodstream; it is capable of reacting with the localized metal complex of #1 to generate locally toxic oxidative species; it is not overly toxic in and of itself.

6) as an alternative to #1 above but incorporating the delivery concept I propose substituting the bone seeking targeting strategy for a targeting process utilizing molecular recognition such that the metal complex is covalently bound to a tumor and receptor recognizing molecule. An example would be attachment of the metal complex to a monoclonal antibody that binds to and stays on the outer surface of tumor cells or cells associated primarily with the disease (ie angiogenic vascular producing cells near tumors, cancer cells, etc). This way the metal complex can react with blood borne peroxide species to deliver a toxic dose.

7) In order to further enhance the target selectivity of

[Continued]

IDEAS ON ALTERNATIVES TO IONIZING RADIATION

#7) (CONTINUED) of the monoclonal antibody approach Joe Sailer

I also propose making use of the so-called pretarget strategy employed by NEORX using radioactive metal ions. I propose substituting an iron, manganese, or copper complex (not radioactive) for their radioactive complex & using a "cleansing" antibody, or avidin/streptavidin to purify the blood of nonlocalized modified antibody prior to introducing the low molecular weight biotin conjugated Fe/Mn/Cu complex. This use of pretargeting with my concept of metal ions + peroxide would be a way to enhance the delivery of specificity to the process. The last step in the process as I see it is introduction of the peroxide into the bloodstream so that it comes in contact with the target localized reactive metal complex.

8) I also envision #7 above with non-antibody cell specific or cell surface antigens seeking agents. For example somatostatin recognizing molecules like octreotide can be covalently attached to the iron/Mn/Cu chelating agents for delivery (selective) to somatostatin positive cells such as found in some tumors. This would then be followed by introducing peroxides into the blood stream after the metal complex not localized at the cell surface had mostly been cleared from the blood stream. Any targeting molecule capable of covalent attachment to the metal complex could be used for the targeting part.

9) I also envision certain Fe/Cu/Mn complexes that themselves could show desirable localization in target cells & thus be useful as described in #1 above concept.

D

PTO/SB/95 (06-1999)
Approved for use through 8/31/2002. OMB 0651-0030
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure Document Dep sit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: Ideas on Tumor Selecting Chelating Agents: Specific Prepared compounds

Enclosed is a disclosure of the above-titled invention consisting of 1 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

328 West Columbine Lane
Address

JOSEPH R. GARLICH
Typed or printed name

Westfield IN 46074

[redacted]
Date

City, State, Zip

NOTICE TO INVENTORS

It should be clearly understood that a I ... ion, nor will its receipt date in any way become the effective filing date of a later ... ent may be relied upon only as evidence of conception of an invention and a pate ... nt protection is desired.

DISCLOSURE DOCUMENT NO.
476824
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

Your Disclosure Document will be retained f ... ent and Trademark Office (PTO) and will be destroyed thereafter unless it is referred to in ... r period. The Disclosure Document may be referred to by way of a letter of transmittal in a ... n a pending application. Unless it is desired to have the PTO retain the Disclosure Docun ... hat it be referred to in the patent application.

The two-year retention period should not be ... the inventor can wait to file his/her patent application without possible loss of benefits. ... invention an affidavit or testimony referring to a Disclosure Document must usually also ... s filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or If you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the PTO. The publication, Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

... ication of your invention anywhere in the world more than one ...

... er examples of evidence which may ...

... ctions of patents and patent-related ... such as General Information Con... the location of the PTDL closest to ... of the Official Gazette, or call the ... To ensure assistance from a PTDL ... and hours.

... Disclosure Document Deposit Requests ... complete, including gathering, preparing ... the individual case. Any comments on ... the Chief Information Officer, U.S. ... EES OR COMPLETED FORMS TO THIS ...

JOSEPH R. GARLICH, PH.D
DAWN GARLICH
328 COLUMBINE LN.
WESTFIELD, IN 46074

6349

Date [redacted]

20-7380/2740

PAY TO THE ORDER OF Commissioner of Patents + Trademarks $ 10 — no/100

Ten and no/100 DOLLARS

DOW EMPLOYEES CREDIT UNION
... STREET AND ... ROAD
BOX 81048
INDIANAPOLIS, IN ...

FOR Disclosure Document Joseph R. Garlich

[redacted]

Rdek

[redacted]

PTO/SB/95 (06-1999)
Approved for use through 8/31/2002. OMB 0651-0030
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure Document Deposit Request

Mail to:
Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R. Garlich

Title of Invention: IDEAS ON TUMOR SELECTIVE CHELATING AGENTS: SPECIFIC PREPARED COMPOUNDS

Enclosed is a disclosure of the above-titled invention consisting of 1 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10.00 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich
Signature of Inventor

328 West Columbine Lane
Address

JOSEPH R. GARLICH
Typed or printed name

Westfield IN 46074
City, State, Zip

[redacted]
Date

NOTICE TO INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the Patent and Trademark Office (PTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the PTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the PTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

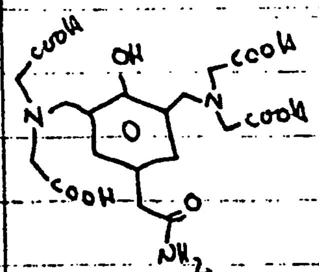
There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related reference materials available to the public, including automated access to PTO databases. Publications such as *General Information Concerning Patents* are available at the PTDLs, as well as the PTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the PTO's Web site or in every issue of the Official Gazette, or call the PTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To ensure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

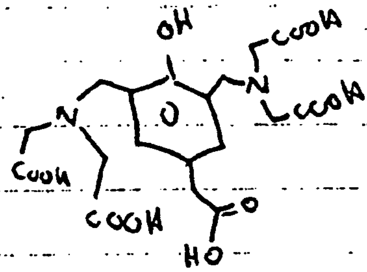
Joseph R. Garlich

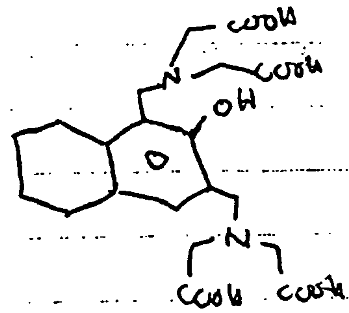
IDEAS ON TUMOR SELECTIVE CHELATING AGENTS Joseph R Garlich

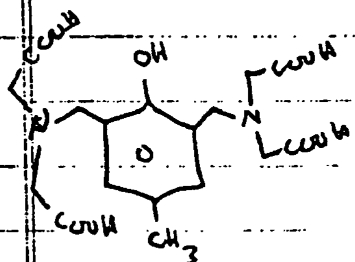
[redacted] —Specific Prepared Structures Page 1 of 1

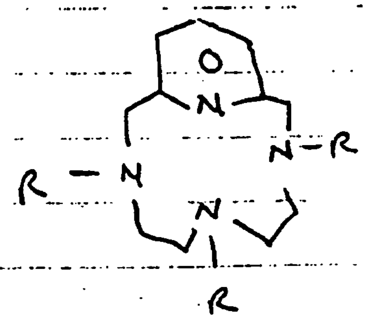
Below are new compositions which may have the ability to deliver metal ions selectively to tumor cells or other diseased cells (preferentially over healthy cells) in mammals:

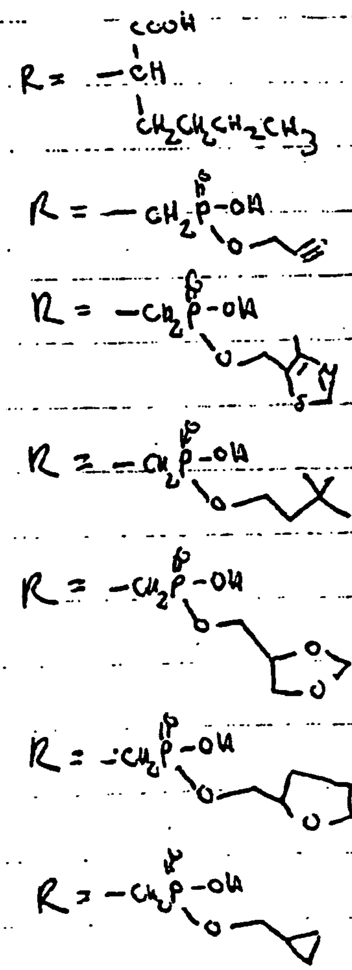


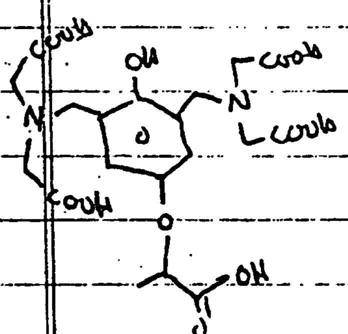


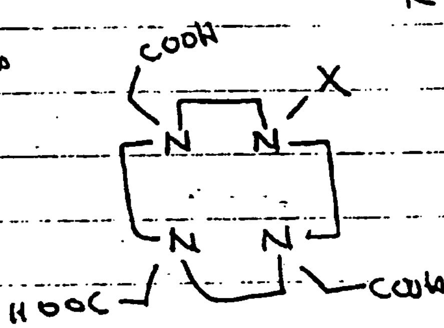


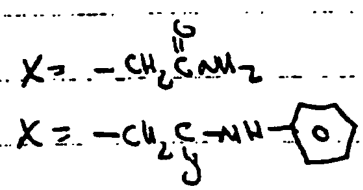


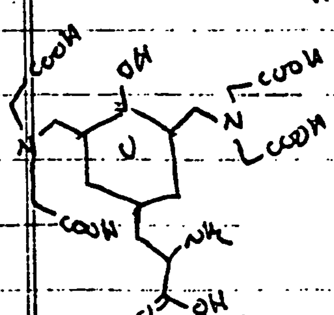












(Tyrosine based)

The above chelating agents complexed with +2 & +3 valent metal ions are also new compositions especially Fe^{+3}, Gd^{+3}, Sm^{+3}, In^{+3}, Ga^{+3}, Ho^{+3}

OIPE
FEB 1 2 2001
PATENT & TRADEMARK OFFICE

DD $

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Disclosur Docum nt Deposit Request

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R Garlich

Title of Invention: Ident. and selective in vivo localization of metal ligand complexes and the use of metal-ligand complexes in lead generation

Enclosed is a disclosure of the above-titled invention consisting of 3 sheets of description and 6 sheets of drawings. A check or money order in the amount of $10 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich	328 West Columbine Lane
Signature of Inventor	Address
Joseph R. Garlich	Westfield IN 46074
Typed of printed name	
[redacted]	
Date	City, State, Zip

NOTICE OF INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date ... effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evide... invention and a patent application should be diligently filed if patent protection is desired.

...tes ... withi... by a ... the two...

...an wait ... an a ... filing th...

... practic... under the patent ... agent registered to practice ...tes Patent and Trademark ...d agents are also listed in ...ich may be consulted.

...n anywhere in the world ... of a patent on it.

...er examples of evidence

DISCLOSURE DOCUMENT NO. 488590
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

DAWN L. GARLICH 02/99
JOSEPH R. GARLICH
328 W COLUMBINE LANE, 317-581-1635
WESTFIELD, IN 46074

210
20-6/740 370

[redacted] DATE

PAY TO THE ORDER OF Commissioner of Patent & Trademark $ 10 — no/100

Ten and no/100 DOLLARS

NationalCity.
National City Bank of Indiana
Indianapolis, Indiana

VALUEPAK

FOR Applica... metal complexes Joseph R Garlich

[redacted]

... of patents and patent-related reference materials available to the public, including automated access to USPTO databases. Publications such as *General Information Concerning Patents* are available at the PTDLs, as well as the USPTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the USPTO's Web site or in every issue of the Official Gazette, or call the USPTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To insure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted]

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosur Document Deposit R qu st

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R Garrick

Title of Invention: Means and selective in vivo localization of metal ligand complexes and reuse of metal-ligand complexes w Lead Generation

Enclosed is a disclosure of the above-titled invention consisting of 3 sheets of description and 0 sheets of drawings. A check or money order in the amount of $10 is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garrick
Signature of Inventor

Joseph R. Garrick
Typed of printed name

[redacted]
Date

328 West Columbine Lane
Address

Westfield IN 46074

City, State, Zip

NOTICE OF INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the United States Patent and Trademark Office (USPTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the USPTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the USPTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

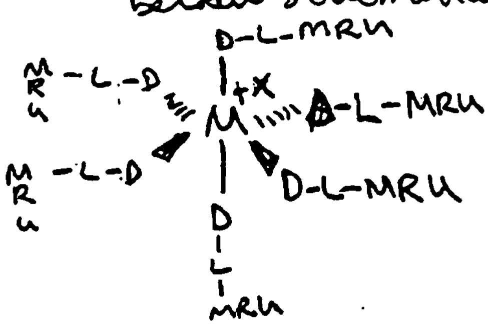
Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related reference materials available to the public, including automated access to USPTO databases. Publications such as *General Information Concerning Patents* are available at the PTDLs, as well as the USPTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the USPTO's Web site or in every issue of the Official Gazette, or call the USPTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To insure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted] Joseph R Garlich (Joseph R. Garlich) Page 1 of 3

IDEAS ON SELECTIVE in vivo LOCALIZATION OF Metal-Ligand Complexes and the use of metal-ligand complexes in lead generation.

There is a great need for new strategies to find lead compounds to screen & optimize against biological targets to produce useful drugs. Currently to produce lead compounds or even screening libraries of compounds a chemist has to prepare an organic scaffold & then substitute various groups on it with moieties hopefully capable of binding to the target site with specificity and selectivity and potency. An example of such as scaffold is the Diazapine nucleus in combinatorial chemistry. I propose using metal-ligand complexes as screening libraries to find the right spatial arrangement of target binding moieties (molecular recognition units) to give desired selectivity & potency. The complexes thus found could be active drugs themselves or thru molecular modeling a purely organic equivalent to give the same spatial arrangement of the molecular recognition units (MRU) can be identified. This concept is shown below schematically {for an octahedral complex - others may be simpler!)



Structure I

when M = Metal ion

X = positive valence of metal ion

D = electron donating group coordinated to metal ion

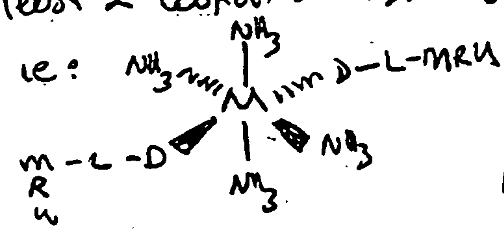
L = an organic linker attaching the electron donating group "D" with the MRU

MRU = Molecular recognition group that binds or "recognizes" the target protein, cell wall, receptor membrane, etc.

[redacted] Joseph R Garlich

IDEA's (Cont)

It is also envisioned that it's not ~~neccesary~~ necessary for all coordination sites of the metal ion to be thus occupied In order for it to be useful for example I propose a minimum of at least 2 coordination sites are occupied by the -D-L-MRU group ie:



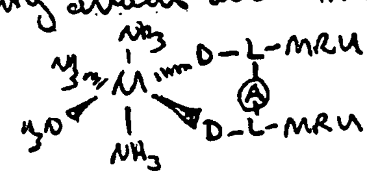
where the amine groups do not necessarily participate in the binding to the target site.

I also envision the above where the metal ion is one that imparts pharmaceutical value in addition to providing a scaffold for "MRU"'s such as but not limited to ① radioactive metals such as Cu, Rh, Ho, Sm, Lu, Re, Co, IN, Ga, Tc, Sn, Fe, Sr, etc. for imaging or therapy or both.

② MRI active metal ions - Gd, Fe, Mn etc

③ toxic metals upon internalization by target cell & metabolized (ie Fe, Gd)

It is also envisioned that in order to enhance the performance of the metal complexes as drugs themselves it may be useful to covalently attach one or more "D-L-MRU" groups together such as:



where Ⓐ is the covalent attachment between the arms & is an appropriately sized organic linker.

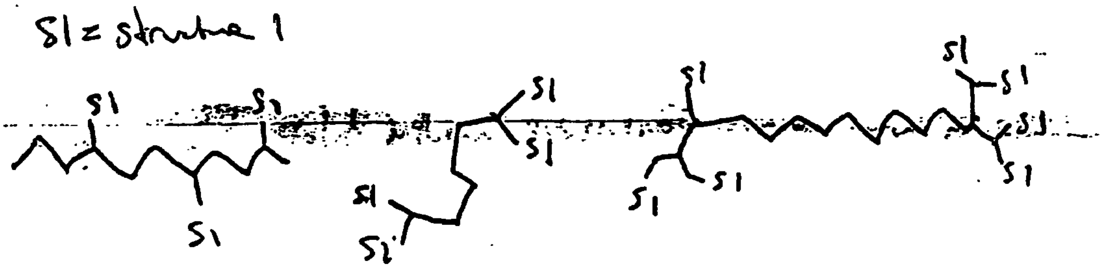
It is also envisioned that the "MRU" groups in all applications described here in could all be identical within one complex but that for combinatorial purposes the "MRU" groups could differ from one another in order to present a diverse collection of spatially arranged "MRU" groups to find the best target binder.

[redacted] Joseph R Sauk

IDEA's (CONT)

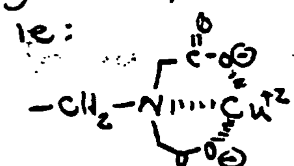
It is also envisioned that multivalent constructs employing two or more of structure I strung together ie:

SI = structure 1



where () is covalent organic linkages; polypeptide or polymer or mixed repeating units, sugars, amides, esters etc.

It is also envisioned that some of the MRU groups could be metal-ligand complex moieties themselves possessing some degree of unfilled coordination sphere such that they would bind very strongly with electron donating groups of the target; ie:

MRU =  (ie Cu-iminodiacetic acid which can bind histidine moieties tightly)

In these cases the metal ion of the MRU could be pharmaceutically valuable as described herein above.

448107

FILING FEE: $10.00
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION

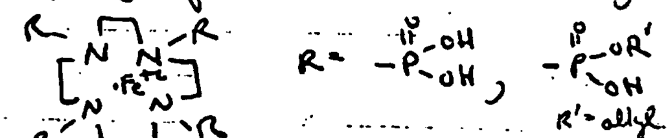
PTO-1652 (4/96)

[redacted]

I propose treating diseases such as Cancer by administering hydrogen peroxide or other similar highly oxidized species to a patient so that the administered oxidant is present to some extent in the bloodstream after the patient has been previously treated by an of the following:

1) A boneseeking iron or manganese complex which is capable of catalyzing the conversion of hydrogen peroxide to cell-damaging radicals.

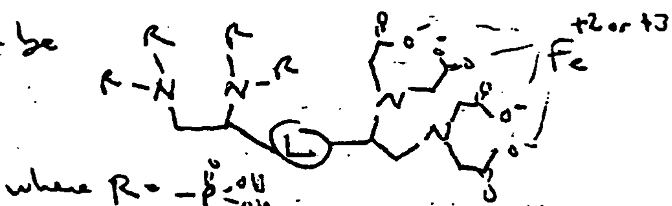
An example would be →



or salts thereof

2). a boneseeking agent that is covalently attached to an iron or manganese complex which is capable of catalyzing the conversion of hydrogen peroxide to cell-damaging free radicals

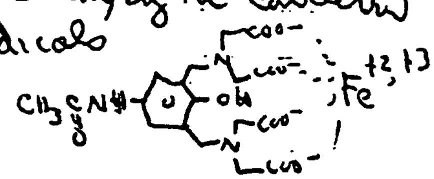
an example would be



or salts thereof and (L) is a covalent attachment (also could be attached thru or in place of the R group)

3) A tumor seeking small molecule containing an iron or manganese complex that is capable of catalyzing the conversion of hydrogen peroxide to cell-damaging free radicals

An example might be



4) An iron or manganese complex that is capable of catalyzing the conversion of hydrogen peroxide to cell-damaging free radicals that is covalently attached to a polypeptide or monoclonal Antibody (or fragment) to deliver the metal complex selectively to the tumor.

Submitted by: Joseph R. Garlich
Joseph R. Garlich

328 West Columbine Lane
Westfield IN 46074

Dated [redacted]

FORM PTOL-479
(Rev. 5-92)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

DISCLOSURE DOCUMENT RECEIPT NOTICE
(No Duplicate Copy)

Receipt of your Disclosure Document and fee of **$10.00** is acknowledged. Since the Patent and Trademark Office did not receive a duplicate copy of your document, the date of receipt and the Disclosure Document identification number have been stamped on this notice. This date and number should be referred to in any future correspondence regarding this Disclosure Document.

You may obtain a duplicate copy of your Disclosure Document by doing the following:

- Submit a written request to include the Disclosure Document number to:

  Box DD
  Commissioner of Patent and Trademarks
  Washington, D.C. 20231

- Provide a check or money order in the amount of **$25.00** made payable to the Commissioner of Patent and Trademarks.

WARNING

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be destroyed two years after the date it was received by the Patent and Trademark Office unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the Patent and Trademark Office retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his or her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

You are also reminded that any public use or placing on sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the Patent and Trademark Office. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the **Superintendent f Documents, Washingt n, D.C. 20402**. Patent attorneys and agents are also listed in the telephone directories of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

OIPE JC162 [redacted] PATENT & TRADEMARK OFFICE

DD $

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Discl sure Document Deposit Request

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R Garlick
Title of Invention: Novel MRI Agents and Metal-Ligand Complexes

Enclosed is a disclosure of the above-titled invention consisting of 3 sheets of description and ______ sheets of drawings. A check or money order in the amount of ______ is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlick	328 West Columbine Lane
Signature of Inventor	Address
Joseph R Garlick	Westfield IN 46074
Typed or printed name	
[redacted]	
Date	City, State, Zip

NOTICE OF INVENTORS

DISCLOSURE DOCUMENT NO.
490496
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION
PTO-1652 (8/99)

It should be clearly understood that a Disclosure Document is not a patent application, no effective filing date of a later filed patent application. A Disclosure Document may be relie invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received b Office (USPTO) and will be destroyed thereafter unless it is referred to in a related patent The Disclosure Document may be referred to by way of a letter of transmittal in a new pat a pending application. Unless it is desired to have the USPTO retain the Disclosure Doc required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during whi application without possible loss of benefits. It must be recognized that in establishing referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application

practice" under the patent
gent registered to practice
es Patent and Trademark
d agents are also listed in
ich may be consulted.

on anywhere in the world
g of a patent on it.

er examples of evidence

ons of patents and patent-
olications such as General
.uspto.gov. To find out the
PTO's Web site or in every
36-9199) or 703-308-HELP
o visiting to learn about its

re Document Deposit Requests.
including gathering, preparing, and submitting the completed Disclosure Document Deposit Request to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

mailed [redacted]

ac6406

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Disclosure Docum nt Deposit Request

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joseph R Garlick

Title of Invention: Novel MRI Agents and Metal-Ligand Complexes

Enclosed is a disclosure of the above-titled invention consisting of 3 sheets of description and ______ sheets of drawings. A check or money order in the amount of ______ is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlick	328 West Columbine Lane
Signature of Inventor	Address
Joseph R Garlick	Westfield IN 46074
Typed or printed name	
[redacted]	
Date	City, State, Zip

NOTICE OF INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the United States Patent and Trademark Office (USPTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the USPTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the USPTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

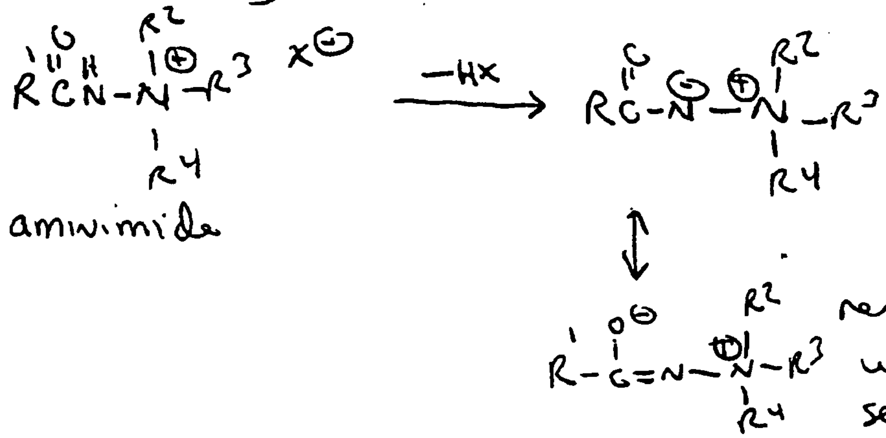
Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related reference materials available to the public, including automated access to USPTO databases. Publications such as *General Information Concerning Patents* are available at the PTDLs, as well as the USPTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the USPTO's Web site or in every issue of the Official Gazette, or call the USPTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To insure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted]

Inventor: Joseph R. Garlich Joseph R Garlich Page 1 of 3
Title: Novel MRI Agents and Metal-Ligand Complexes

MRI agents are usually metal complexes that effect the relaxivity of water molecules in the innersphere of coordination or outer sphere or most preferably with water outside of the outer sphere. Aminimides are acidic molecules that readily lose a proton to generate an internally satisfied Zwitterion as shown:



resonance form with charge separation

1) I propose incorporation of aminimide groups into chelating agents and resulting metal complexes to impart greater water solubility without increasing osmolarity and/or such that the aminimide group interacts (thru N lone pair of electrons or oxygen lone pair of electrons) with the metal ion.

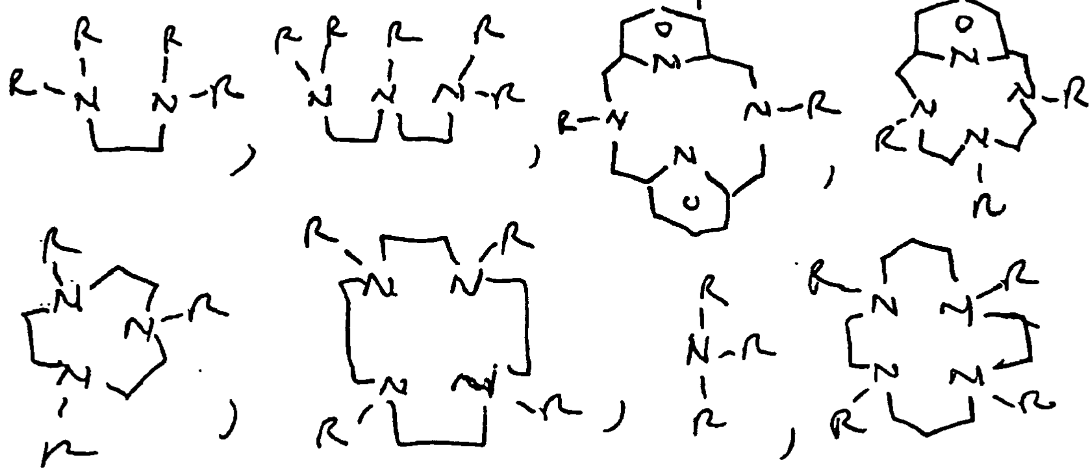
2) An example of 1) could be as listed below (but not limited to:

(these can be useful for MRI imaging, X-ray contrast agents, and nuclear medicine imaging or therapeutic purposes

[redacted]

Inventor: Joseph R. Garlich Joseph R Garlich Page 2 of 3
Con't (Novel MRI Agents...)

depending on what metal ion the chelant is complexed with; also various salts & stages of deprotonation are envisioned



where most of the R groups are selected from usual complexing appendages or H such as but not limited to: $-CH_2COOH$, $CH_2\overset{O}{C}NHOH$, $-CH_2\overset{O}{C}NH_2$, $-$[ring with OH OH], $-CH_2\overset{O}{\underset{}{P}}(OH)_2$,

$-CH_2\overset{O}{P}(OH)(OR')$, $-CH_2\overset{O}{P}(OH)(R')$ where R' is an organic nonchelating moiety & the $\underline{CH_2}$ can be substituted

<u>and</u> at least one R group contains an aminimide group such as:

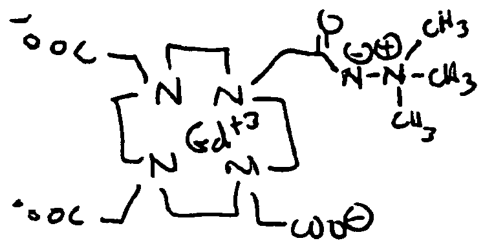
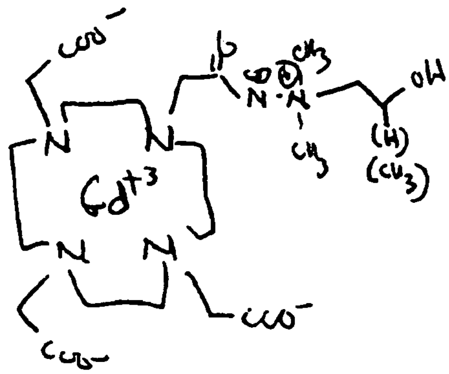
$\{-CH_2\overset{O}{C}\overset{\ominus}{N}-\overset{\oplus}{N}(R^1)(R^2)(R^3)$ or $\{-CH_2\overset{O}{P}(OH)-O-(Z)-\overset{O}{C}\overset{\ominus}{N}-\overset{\oplus}{N}-$, or $\{-\underset{(Z)-\overset{O}{C}\overset{\ominus}{N}-\overset{\oplus}{N}R^1R^2R^3}{CH}\overset{O}{C}OH$

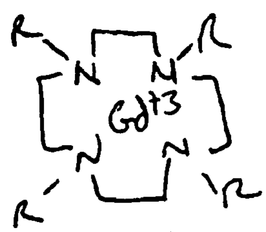
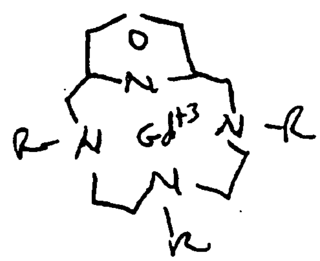
when R^1, R^2, R^3 are organic non hydrogen groups ~~and~~ that go cause a positive charge on the nitrogen to which they are attached and Z group is a low molecular weight (<200 daltons) organic group linking the aminimide to the chelating moiety.

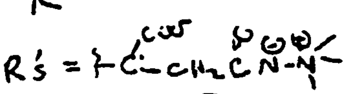
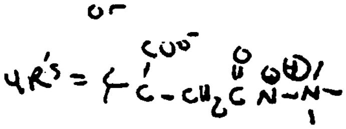
[redacted]

Inventor: Joseph R. Garlich Joseph R Garlich P3 of 3
Con't (Novel MRI agents ...)

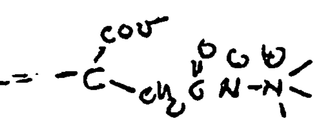
Specific examples which we plan to make & evaluate first are shown below:

 , 

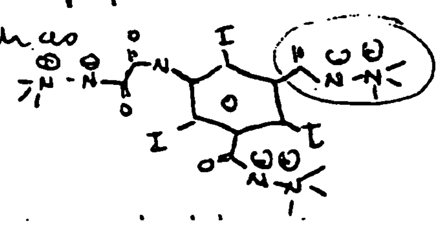
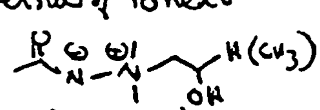
 , 

3R's = 
1R = $-CH_2COO^{\ominus}$
or
all 4R's = 
or
when 3R's as ↑ with
1R = 
or
1R = $-CH_2\overset{O}{\overset{\|}{C}}NH_2$

to give neutral complex w/ 3 aminimide groups

R = 

where CH_2 could be replaced by suitably larger or substituted organic linker & substituents on Nitrogen can be various organic groups to be optimized

I also propose aminimides as part of triiodinated benzene rings such as  = aminimide version of Iohexol or replace  for even more water solubility

... procedures

OIPE JC104
[redacted]
PATENT & TRADEMARK OFFICE

DD/$

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Disclosur Document Deposit Request

Mail to:

Box DD
Assistant Commissioner for Patents
Washington, DC 20231

Inventor(s): Joe Garlich
Title of Invention: IDEAS ON DYNAMIC COMBINATORIAL Libraries USING Metal Complexes

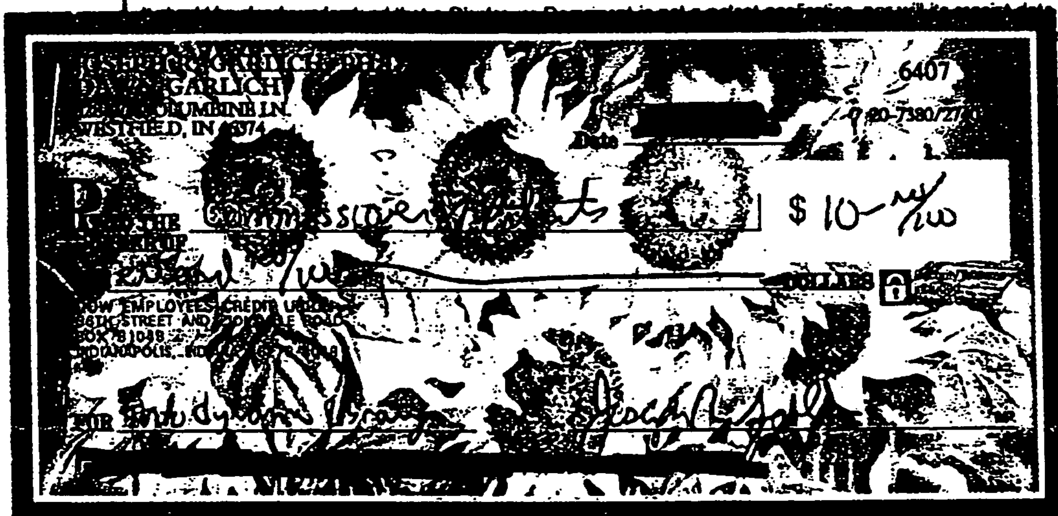
Enclosed is a disclosure of the above-titled invention consisting of 2 sheets of description and ________ sheets of drawings. A check or money order in the amount of __________ is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R. Garlich	328 West Columbine Lane
Signature of Inventor	Address
Joseph R. GARLICH	Westfield IN 46074
Typed of printed name	
[redacted]	
Date	City, State, Zip

NOTICE OF INVENTORS

... in any way become the ... nce of conception of an



... Patent and Trademark ... in the two-year period. ... a separate letter filed in ... two-year period, it is not

... wait to file his/her patent ... n affidavit or testimony ... g the patent application

... actice" under the patent ... t registered to practice ... *Patent and Trademark* ... gents are also listed in ... may be consulted.

... anywhere in the world ... a patent on it.

Disclosures of inventors which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent- ... ublic, including automated access to USPTO databases. Publications such as *General* ... s at the PTDLs, as well as the USPTO's Web site at www.uspto.gov. To find out the ... nsult the complete listing of all PTDLs that appears on the USPTO's Web site or in every ... TO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP ... PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its

DISCLOSURE DOCUMENT NO.
490683
RETAINED FOR 2 YEARS
THIS IS NOT A PATENT APPLICATION

PTO-1652 (8/99)

mailed on March 14, 2001 ... ac 6407

PTO/SB/95 (08-00)
Approved for use through 05/31/2002. OMB 0651-0030
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Disclosure Document D posit Request

Mail to:

Box DD

Assistant Commissioner for Patents

Washington, DC 20231

Inventor(s): Joe Garlich

Title of Invention: IDEAS ON DYNAMIC COMBINATORIAL Libraries Using Metal Complexes

Enclosed is a disclosure of the above-titled invention consisting of 2 sheets of description and ________ sheets of drawings. A check or money order in the amount of ____________ is enclosed to cover the fee (37 CFR 1.21(c)).

The undersigned, being a named inventor of the disclosed invention, requests that the enclosed papers be accepted under the Disclosure Document Program, and that they be preserved for a period of two years.

Joseph R Garlich — 328 West Columbine Lane

Signature of Inventor — Address

Joseph R. GARLICH — Westfield IN 46074

Typed of printed name

[redacted]

Date — City, State, Zip

NOTICE OF INVENTORS

It should be clearly understood that a Disclosure Document is not a patent application, nor will its receipt date in any way become the effective filing date of a later filed patent application. A Disclosure Document may be relied upon only as evidence of conception of an invention and a patent application should be diligently filed if patent protection is desired.

Your Disclosure Document will be retained for two years after the date it was received by the United States Patent and Trademark Office (USPTO) and will be destroyed thereafter unless it is referred to in a related patent application filed within the two-year period. The Disclosure Document may be referred to by way of a letter of transmittal in a new patent application or by a separate letter filed in a pending application. Unless it is desired to have the USPTO retain the Disclosure Document beyond the two-year period, it is not required that it be referred to in the patent application.

The two-year retention period should not be considered to be a "grace period" during which the inventor can wait to file his/her patent application without possible loss of benefits. It must be recognized that in establishing priority of invention an affidavit or testimony referring to a Disclosure Document must usually also establish diligence in completing the invention or in filing the patent application since the filing of the Disclosure Document.

If you are not familiar with what is considered to be "diligence in completing the invention" or "reduction to practice" under the patent law or if you have other questions about patent matters, you are advised to consult with an attorney or agent registered to practice before the USPTO. The publication, *Attorneys and Agents Registered to Practice Before the United States Patent and Trademark Office*, is available from the Superintendent of Documents, Washington, DC 20402. Patent attorneys and agents are also listed in the telephone directory of most major cities. Also, many large cities have associations of patent attorneys which may be consulted.

You are also reminded that any public use or sale in the United States or publication of your invention anywhere in the world more than one year prior to the filing of a patent application on that invention will prohibit the granting of a patent on it.

Disclosures of inventions which have been understood and witnessed by persons and/or notarized are other examples of evidence which may also be used to establish priority.

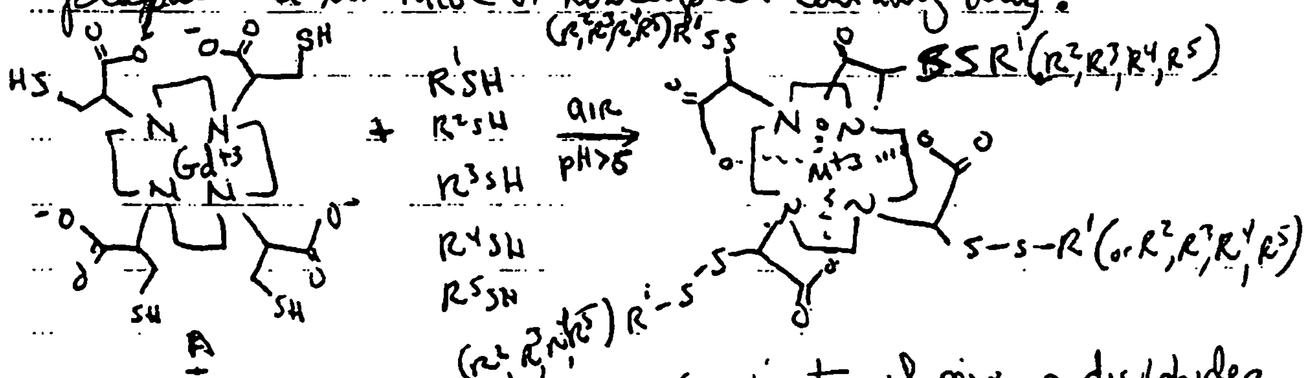
There is a nationwide network of Patent and Trademark Depository Libraries (PTDLs), which have collections of patents and patent-related reference materials available to the public, including automated access to USPTO databases. Publications such as *General Information Concerning Patents* are available at the PTDLs, as well as the USPTO's Web site at www.uspto.gov. To find out the location of the PTDL closest to you, please consult the complete listing of all PTDLs that appears on the USPTO's Web site or in every issue of the Official Gazette, or call the USPTO's General Information Services at 800-PTO-9199 (800-786-9199) or 703-308-HELP (703-308-4357). To insure assistance from a PTDL staff member, you may wish to contact a PTDL prior to visiting to learn about its collections, services, and hours.

[redacted]

Inventor: Joe Garlich Joseph R Garlich page 1 of 2

Title: Ideas on dynamic combinatorial Libraries using metal complexes.

A recent report (JACS 2000 p12063-12064) describes the use of dithiols in mixtures to generate a dynamic combinatorial library of cyclic disulfides. I propose using a metal ligand complex as the backbone or template for a thiol exchange combinatorial library generation to prepare novel compositions of matter to be evaluated for drug use. This is shown schematically for a DOTA based system but would be applicable to other complexes such that one can evaluate the library against a target (biological) to find the right combination of spatially oriented groups to bind to and agonize or antagonize the biological receptor. This compound can then be used as a drug or optimized to produce a drug or serve as a model to prepare a nonthiol or noncomplex containing drug:



A

combinatorial mix of disulphides (lower pH to stop dynamic interconversions)

Expose dynamic library to target → biological target binds best combination → analyze by M.S. (mass spec) to determine identity

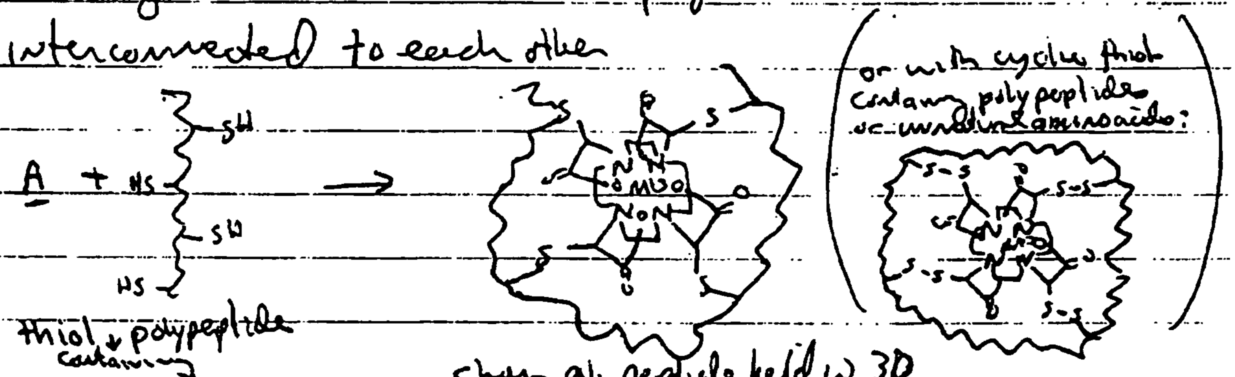
[redacted]

Inventor: Joe Garlich Joseph R Garlich page 2 of 2

Title: IDEAS ON DYNAMIC combinatorial libraries ...

Continued:

The $R^1SH \ldots R^5SH$ can be various organothiols so long as they are capable of forming disulfide linkages. Of special interest is when the RSH is a peptide or polyamide containing cysteine (RSH) groups such that when incorporated into 3D space on a complex it presents a certain stable presentation of peptides for molecular recognition of a target protein, receptor, membrane, molecule or cell. The RSH's can be single entities as shown on page 1 or could be interconnected to each other



shows polypeptide held in 3D Spatial arrangement*

The substitution of the complex with thiol groups is shown on the acetate arms but could be on the chelator backbone (between nitrogens) or a mixture of both

The metal ion can be chosen to be diagnostic medical utility (such as Gd^{+3} for MRI) or therapeutic medical utility (such as radioactive lanthanides, Y-90 or nonradioactive toxic agents such as Fe^{+3}).

*as described for monovalent thiols this would be an equilibrium at higher pH's but frozen at lower pH to find one member that recognizes the target.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/032289

International filing date: 30 September 2004 (30.09.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/507,427
Filing date: 30 September 2003 (30.09.2003)

Date of receipt at the International Bureau: 17 November 2004 (17.11.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse